

## A REVISION OF THE GEOGRAPHICAL DISTRIBUTIONS OF THE SOUTHEAST ASIAN SHREWS (*Crocidura dracula* & *C. fuliginosa*) BASED ON NEW COLLECTION IN VIETNAM

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**Abstracts:** The Southeast Asian Shrews (*Crocidura dracula* & *C. fuliginosa*) are so similar in external morphology and skull characters. Thus, their taxonomic status and distribution have debated for a long time. Recent researches provided the molecular identification which caused the change of outlook on occurrence area of these two species. Based on new collections, we have updated the geographical distribution of *C. dracula* and *C. fuliginosa* in Southern China and Southeast Asia (SEA) by using morphology and molecular approaching. This study also suggested that the Mekong River is the natural barrier of these species.

**Keywords:** Barrier, biogeography, white-toothed shrews, Mekong River.

### 1. INTRODUCTION

Vietnam is located in a subtropical-tropical transition zone having complex climate and on the eastern margin of the Indochinese Peninsula with a diverse topography including many islands on the East Sea. Because of its location, topography and climate, the long country experiences a high species richness of mammals (Sterling and Hurley 2005; Sterling et al., 2006; Can et al., 2008). According to the recent checklist, Vietnamese mammalian fauna consists more than 295 species of 114 genera, 37 families, and 13 orders of mammals (excluding marine mammals) (Dang Ngoc Can et al., 2008).

The white-toothed shrews *Crocidura* is the speciose genus of mammals with 198 currently recognized species and these animals are widely distributed from Africa to Southeast Asia as far as islands to the east of Sulawesi via crossing Europe and the Palearctic (Jenkins et al., 2009; Burgin and He, 2018). Recent studies have shown a great diversity of the genus *Crocidura* in Vietnam by a list of 15 species that have been recorded along the country (Bui Tuan Hai et al., 2019).

*C. dracula* and *C. fuliginosa* are distinct species with very similar external morphology and skull characters, hence the taxonomic status of these species has been considered for a long time. *C. fuliginosa* was firstly recorded from Myanmar as a new species by Blyth (1855), while *C. dracula* was originally described by Thomas (1912)

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from Southern Yunnan (China) and their distribution ranged throughout southern China to adjacent Indochina (Allen, 1938; Ellerman and Morrison-Scott, 1951). However, *C. dracula* was noted as a synonym or subspecies, *C. f. dracula* by many authors (Jenkins, 1976; Heaney and Timm, 1983; Dang Huy Huynh et al., 1994; Kuznetsov, 2006; Jiang and Hoffmann, 2001; Hutterer, 2005; Dang Ngoc Can et al., 2008 and Jenkins et al., 2009). Dubey et al. (2008) detected the nuclear genetic differences of *C. fuliginosa* sensu lato between Yunnan (China) and Peninsular (Malaysia). Based on the comparative study of mtDNA, Bannikova et al. (2011) suggested the distinct taxonomic position of *C. dracula* (from Northern Vietnam and Southern China) and *C. fuliginosa* (Southern Myanmar, Malaysia and Con Son island (Vietnam)) including the first molecular data of *C. fuliginosa* in Vietnam. Moreover, Bui Tuan Hai et al. (2017) found the significant differences in skull morphology between specimens from Hon Khoai island (Ca Mau, Vietnam) and others Vietnamese *C. dracula* populations (from Hoa Binh, Thanh Hoa, Nghe An, Phu Tho and Vinh Phuc) by using multivariate analysis.

The actual distribution of *C. dracula* and *C. fuliginosa* in Vietnam is still questionable (Abramov et al., 2012, 2013 and Bui Tuan Hai et al., 2017). Before the DNA revision of Bannikova et al. (2011), many researches claimed *C. fuliginosa* being widespread in the mainland of Vietnam (Heaney & Timm, 1983; Dang Huy Huynh et al., 1994, Kuznetsov, 2006; Dang Ngoc Can et al., 2008; Jenkins et al., 2009). Later, Abramov et al. (2013) and Bui Tuan Hai et al. (2017) discussed that *C. dracula* is distributed in northern India, Southern China, Myanmar and Northern Vietnam (approached to Nghe An). Indeed, the distribution of *C. dracula* (or *C. fuliginosa*) in India has not been confirmed by specimens. Besides, Abramov et al. (2012, 2013, 2018) and Bui Tuan Hai et al. (2017) also mentioned the occurrence of *C. fuliginosa* in Con Son islands (Ba Ria - Vung Tau, Vietnam) and Hon Khoai island (Ca Mau, Vietnam), respectively. The current IUCN distribution map (Fig. 1A; Molur, 2016) showed the wild appearance of *C. fuliginosa* have been revised by this study. Here we have updated the geographical distribution of *C. dracula* and *C. fuliginosa* in Southern China and SEA.

## 2. MATERIALS AND METHODS

A total 52 specimens of *Crocidura*, which were collected from 13 localities (Dien Bien, Lai Chau, Son La, Lao Cai, Hoa Binh, Ha Giang, Phu Tho, Vinh Phuc, Thanh Hoa, Nghe An, Ba Ria-Vung Tau, Ca Mau and Kien Giang), were used in this study. The morphological voucher specimens were deposited in Vietnam National Museum of Nature and Institute of Ecology and Biological Resources (Vietnam Academy of Science and Technology), Zoological Museum of Saint Petersburg Zoological Institute (Russian Academy of Sciences), and Smithsonian National Museum of Natural History (U.S.A).

### Morphological identification

The species identification was based on external morphology and skull characters followed Blyth (1855), Thomas (1912), Jenkins et al. (2009), Bannikova et al. (2011), Bui Tuan Hai et al. (2017) and Burgin and He (2018).

### Molecular data and phylogenetic analyses

DNA extraction and amplification based on the protocols of Kuraishi et al. (2013), modified by Nguyen et al. (2015) and referred the thermocycling procedure by Bannikova et al. (2011). The complete mitochondrial cytochrome *b* gene (*Cytb*) was amplified by PCR with the primers as “SoriR: TGACATGAAAAATCATCGTTG SoriF: CCATCTCTGGTTTACAAGAC”. The PCR products were temporarily preserved at -20°C storage and were sequenced by First BASE (Malaysia). The sequencing results were molecularly identified for species by blasting on GenBank. Chromas Pro software (Technelysium Pty Ltd., Tewantin, Australia) and MEGA X (Kumar et al., 2018) were used to edit and align the sequences. The best-fit model for alignment was selected by jModeltest2 (Darriba et al., 2012) based on Akaike Information Criterion (AIC) as GTR+I+G. Phylogenetic trees were constructed by using maximum likelihood (ML) and Bayesian inference (BI) via Kakusan 4 (Tanabe, 2011).

We conducted a DNA analysis of a total 25 sequences. Among them, we also included 10 *cytb* sequences data from GenBank which were published in earlier studies (by Motokawa et al., 2004; Esselstyn et al., 2009; Esselstyn and Oliveros, 2010; Bannikova et al., 2011 and Guo et al., 2011). GenBank accession numbers of sequences used in our analysis including AB175079, GU981271, GU358522, JX18194, FJ813925, JF784171, AB115557, AB175083, AB175085, JX181941.

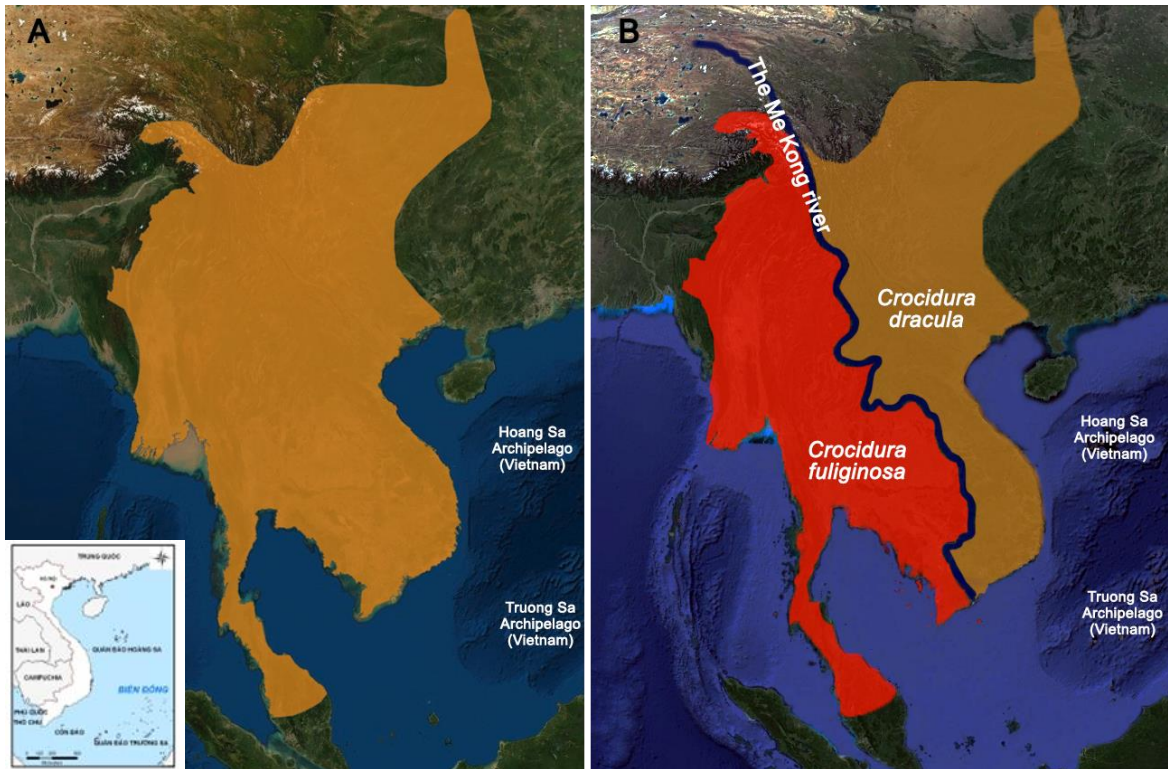
### 3. RESULTS AND DISCUSSION

By comparing external morphology and skull characters, we identified 46 specimens collected from 10 localities in mainland of Vietnam including Dien Bien, Lai Chau, Son La, Lao Cai, Hoa Binh, Ha Giang, Phu Tho, Vinh Phuc, Thanh Hoa, and Nghe An as *C. dracula*. Other 6 specimens collected from Ba Ria-Vung Tau (Con Son island, 2 vouchers), Ca Mau (Hon Khoai island, 3 vouchers), and Kien Giang (Tho Chu island, 1 voucher) belonged to *C. fuliginosa*.

In Bayesian inference statistic, the posterior probability values were congruent with maximum likelihood bootstrap support. The ML and BI analyses generated the trees with the same overall topology. Thus, the ML tree (Fig. 2) with a clearly designed was used to show the genetic relationship among studied objects. The phylogenetic result indicated that the specimens, which were collected in Nghe An, Thanh Hoa, Vinh Phuc, Ha Giang, Hoa Binh, Lai Chau, Dien Bien, Son La, belonged to clade A with *C. dracula* from Yunan (China). Meanwhile, the specimens from the islands Tho Chu and Hon Khoai (Vietnam) were nested in the clade B together with the specimens were reported in Peninsula (Malaysia), Kampot (Cambodia) and Con Son island (Vietnam) by Esselstyn et al. (2009) and Bannikova et al. (2011). Proportional (*p*) distances of *cytb* compare two lineages A and B were from 9.83% to 10.03% with a perfect probability of bootstrap support. Other clades (C, D and E) were presented for making well topology of phylogenetic tree and clarification of relationship among *Crocidura* species.

This study has confirmed that *C. dracula* and *C. fuliginosa* have been not sympatric in SEA. *C. dracula* distributed from Southern China throughout Laos, a part of Cambodia

and recently extended limited to Nghe An province (mainland of VN). Although, according to our research, *C. fuliginosa* has not been found in continental Vietnam, this species has occurred only in several islands mentioned above. However, in association among this study and Esselstyn et al. (2009), Bannikova et al. (2011), Abramov et al. (2012, 2013, 2018) and Bui Tuan Hai et al. (2017, 2019), *C. fuliginosa* distributed apparently from Myanmar to Thailand, Cambodia, Peninsula (Malaysia) and Southernmost Vietnam including some coastal islands (Fig. 1B).



**Fig. 1. A. Distribution of *Crocidura fuliginosa* by the IUCN. B. Distribution of *C. dracula* and *C. fuliginosa* by this study**

The absence of *C. fuliginosa* on the Vietnamese mainland may be due to human impact on the habitat of this species during the exploitation of the Mekong Delta and the influence of saltwater intrusion. Nevertheless, *C. fuliginosa* is still able to expand the distribution on the mainland of Vietnam in the east of Mekong River.

Esselstyn et al. (2009) indicated that the lineage divergence of the most recent common ancestor to “*C. fuliginosa*” (in fact, they are two specimens of *C. fuliginosa* in Malay Peninsula and one specimen of *C. dracula* from Ha Giang, Northern Vietnam) was taken place in nearly 20 mya. In contrast, by the sedimentological, thermochronological and tectonic information, Nie et al. (2018) suggested that the Mekong River have started to drain since about 17 mya during the middle Miocene. Moreover, *Crocidura* shrews were reported that their bodies remain relatively unspecialized with no limbs adaptations for swimming (Hutterer, 1985, Bugin & He, 2018). Therefore, we speculated that the most

recent common ancestor of *C. fuliginosa*-*C. dracula* had dispersed and was widely distributed in Southern China and SEA. The two populations then diverged due to the influence of geographical isolation caused by the rising history of the Mekong River basin.

In the light of the above arguments, we suggest that *C. dracula* distributes on the Eastside of the Mekong river while *C. fuliginosa* appears on the opposite direction and the Mekong River is the natural barrier of these two species.

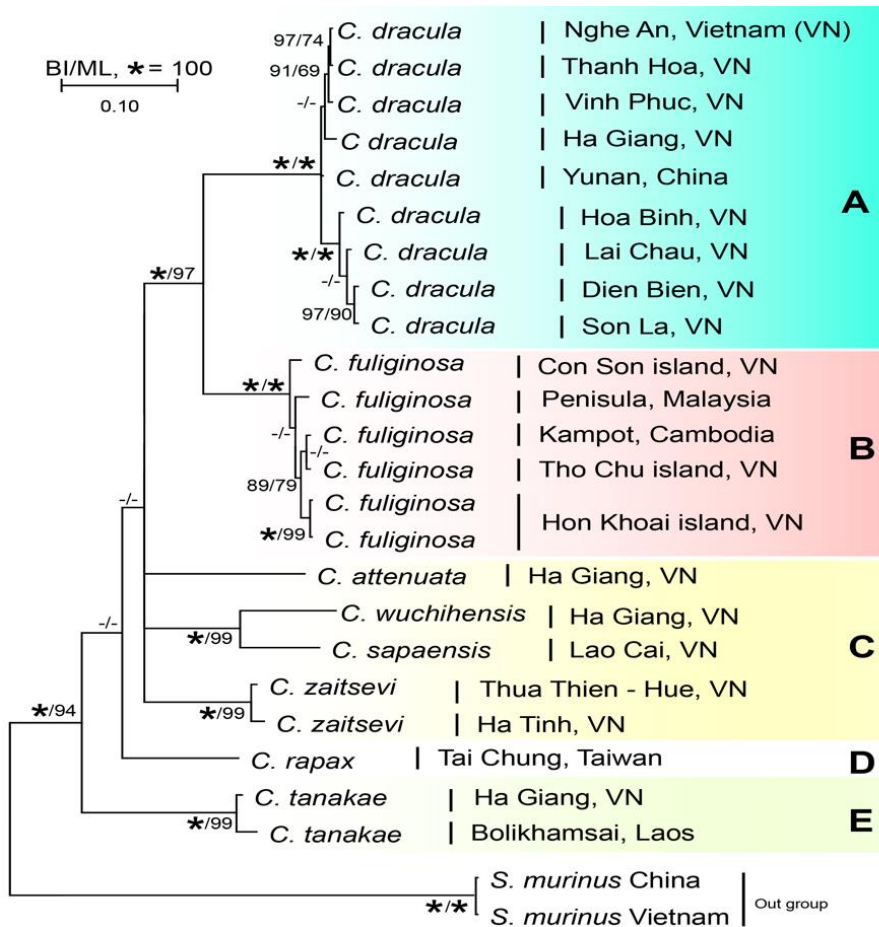


Fig. 2. ML tree based on *Cytb* of *Crocidura* spp.. Numbers near the branches represent bootstrap support (BS)

#### 4. CONCLUSION

Our results suggested that *C. dracula* and *C. fuliginosa* are allopatric species within Southern China and Southeast Asia. *C. dracula* was distributed in China, Laos, and Vietnam limited to the East bank of the Mekong River. On the contrary, *C. fuliginosa* occurred in the westside of Mekong River throughout Malay Peninsula and Myanmar including the Vietnamese southernmost mainland and coastal islands, Cambodia, and Thailand. The speciation of these two sister species may relate to geographical isolation by the Mekong River.

Additionally, further research on the occurrence of *C. dracula* and *C. fuliginosa* in India, Malaysian Borneo and Indonesia is needed to provide a good conservation status.

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## REFERENCES

- Abramov A. V., Dang Ngoc Can, Bui Tuan Hai & Nguyen Truong Son, 2013. An annotated checklist of the insectivores (Mammalia, Lipotyphla) of Vietnam. *Russian Journal of Theriology*, 12(2): 57-70.
- Abramov A. V., Kruskop S. V. & Shchinov A. V., 2018. Mammals of Con Son Island, Southern Vietnam. *Russian Journal of Theriology*, 17(1): 1-16.
- Abramov A. V., Bannikova A. A., Rozhnov V. V., 2012. White-toothed shrews (Mammalia, Soricomorpha, *Crocidura*) of coastal islands of Vietnam. *Zookeys*, 207: 37-47.
- Allen G. M., 1938. The Mammals of China and Mongolia. American Museum of Natural History, New York, 620 p.
- Bannikova A. A., Abramov A. V., Borisenko A. V., Lebedev V. S. & Rozhnov V. V., 2011. Mitochondrial diversity of the white-toothed shrews (Mammalia, Eulipotyphla, *Crocidura*) in Vietnam. *Zootaxa*, 2812: 1-20.
- Blyth E., 1855. Report for May Meeting 1855. *Journal of the Asiatic Society of Bengal*, 24(4): 359-363.
- Bui Tuan Hai, Ly Ngoc Tu, Vu Thuy Duong, Nguyen Truong Son, 2017. Geographic variation in skull size and shape of *Crocidura dracula* (Mammalia: Soricidae) in Vietnam. *Proceedings of the 7th National Scientific Conference on Ecology and Biological Resources*: 670-677.
- Bui Tuan Hai, Ly Ngoc Tu, Vu Thuy Duong, Le Duc Minh, Nguyen Thi Tham, Nguyen Truong Son, 2019. Supplementary data of Insectivores (Mammalia, Eulipotyphla) in Vietnam. *Journal of Biology*, 41 (2se1&2se2): 393- 407.
- Burgin J. E., He K., 2018. Family Soricidae (Shews). In: Wilson, D. E. & Mittermeier, R. A. (ed). *Handbook of the Mammals of the World. Vol. 8. Insectivores, Sloths and Colugos*, Lynx Edicions, Barcelona, pp. 332-51.
- Dang Ngoc Can, Endo H., Nguyen Truong Son, Oshida T., Le Xuan Canh, Dang Huy Phuong, Lunde D. P., Kawada S. -I., Hayashida A., Sasaki M., 2008. Checklist of Wild Mammal Species of Vietnam, Ha Noi: Primate Research Institute, Inuyama, Japan & Institute of Ecology and Biological Resources, 400 p. (in Vietnamese).
- Dang Huy Huynh, Dao Van Tien, Cao Van Sung, Pham Trong Anh & Hoang Minh Khiem, 1994. Checklist of Mammals in Vietnam. Ha Noi: Publishing House "Science and Technics", 168 p. (in Vietnamese).
- Darriba D., Taboada G. L., Doallo R., Posada D., 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods*, 9(8): 772.

- Dubey S., Salamin N., Ruedi M., Barriere P., Colyn M., Vogel P., 2008. Biogeographic origin and radiation of the Old World crocidurine shrews (Mammalia: Soricidae) inferred from mitochondrial and nuclear genes. *Molecular Phylogenetics and Evolution*, 48: 953-963.
- Ellerman J. R., Morrison-Scott T. C. S., 1951. Checklist of Palaearctic and Indian Mammals, 1758 to 1946. Trustees of the British Museum (Natural History), UK, 810 p.
- Esselstyn J. A. and Oliveros C. H., 2010. Colonization of the Philippines from Taiwan: a multi-locus test of the biogeographic and phylogenetic relationships of isolated populations of shrews. *Journal of Biogeography*, 37: 1504-1514.
- Esselstyn J. A., Timm R. M. & Brown R. M., 2009. Do geological or climatic processes drive speciation in dynamic archipelagos? The tempo and mode of diversification in Southeast Asian shrews. *Evolution*, 63: 2595-2610.
- Guo W. P., Lin X. D., Wang W., Zhang X. H., Chen Y., Cao J. H., Ni Q. X., Li W. C., Li M. H., Plyusnin A., Zhang Y. Z. 2011. A new subtype of Thottapalayam virus carried by the Asian house shrew (*Suncus murinus*) in China. *Infection, Genetics and Evolution*, 11(8): 1862-1867.
- Heaney L. R. & Timm R. M., 1983. Systematics and distribution of shrews of the genus *Crocidura* (Mammalia: Insectivora) in Vietnam. *Proceedings of the Biological Society of Washington*, 96: 115-120.
- Hutterer R., 1985. Anatomical adaptations of shrews. *Mammal review* 15(1): 43-55.
- Hutterer R., 2005. Order Erinaceomorpha, Order Soricomorpha. In: Wilson D. E. & Reeder D. M. (ed). *Mammals Species of the World. A Taxonomic and Geographic Reference*. Third edition. The Johns Hopkins University Press, US, pp. 212-311.
- Jenkins P. D., Lunde D. P., Moncrieff C. B., 2009. Chapter 10. Descriptions of new species of *Crocidura* (Soricomorpha: Soricidae) from mainland Southeast Asia, with synopses of previously described species and remarks on biogeography. In: Voss R.S. & Carleton M.C. (ed). *Systematic Mammalogy: Contributions in Honour of Guy G. Musser*. *Bulletin of the American Museum of Natural History* 331, US, pp. 356-405.
- Jenkins P. D., 1976. Variation in Eurasian shrews of the genus *Crocidura* (Insectivora: Soricidae). *Bulletin of the British Museum (Natural History), Zoology*, 30: 271-309.
- Jiang X., Hoffmann R. S., 2001. A revision of the white-toothed shrews (*Crocidura*) of southern China. *Journal of Mammalogy* 82: 1059-1079.
- Kumar S., Stecher G., Li M., Knyaz C., and Tamura K., 2018. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution*, 35:1547-1549.
- Kuraishi N., Matsui M., Hamidy A., Belabut D. M., Ahmad N., Panha S., Sudin, A., Yong H. S., Jiang J. P., Ota H., Thong H. T., Nishikawa K. 2013. Phylogenetic and taxonomic relationships of the *Polypedates leucomystax* complex (Amphibia). *Zoological Scripta*, 42(1): 54-70.
- Kuznetsov G. V., 2006. *Mammal of Vietnam*. Moscow: KMK Scientific Press Ltd., 420 p.
- Motokawa M., Harada M., and Lin L. K., 2004. Variation in the Y chromosome of *Crocidura tadae kurodai* (Insectivora, Soricidae). *Mammalian Biology*, 69(4): 273-276.
- Nguyen T. T., Matsui M., Eto K., 2015. Mitochondrial phylogeny of an Asian tree frog genus *Theلودerma* (Anura: Rhacophoridae). *Molecular Phylogenetics and Evolution*, 85: 59-67.

- Nie J., Ruetenik G., Gallagher K., Hoke G., Garziona C. N., Wang W., Stockli D., Hu X., Wang Z., Wang Y, Stevens T., Danišík M., and Liu S., 2018. Rapid incision of the Mekong River in the middle Miocene linked to monsoonal precipitation. *Nature Geoscience*, 11: 944-948.
- Sterling E. J., and Hurley M. M., 2005. Conserving biodiversity in Vietnam: applying biogeography to conservation research. *Proceedings of the California Academy of Sciences* 56 (Suppl. 1) 9: 98-114.
- Sterling E. J., Hurley M. M., and Le D. M., 2006. *Vietnam: A Natural History*. Yale University Press, New Haven and London, 423 p.
- Tanabe A. S., 2011. Kakusan 4 and Aminosan: Two programs for comparing nonpartitioned, proportional and separate models for combined molecular phylogenetic analyses of multilocus sequence data. *Molecular Ecology Resources*, 11: 914- 921.
- Thomas O., 1912. New species of *Crocidura* and *Petaurista* from Yunnan. *Annals and Magazine of Natural History* 8: 686-688.

## **ĐÁNH GIÁ LẠI HIỆN TRẠNG PHÂN BỐ CỦA HAI LOÀI CHUỘT CHÙ RĂNG TRẮNG ĐÔNG NAM Á (*Crocidura dracula* và *C. fuliginosa*) DỰA TRÊN BỘ SƯU TẬP MẪU VẬT MỚI Ở VIỆT NAM**

**Bùi Tuấn Hải<sup>1,3,\*</sup>, Motokawa Masaharu<sup>2</sup>, Ninh Thị Hoà<sup>1,5</sup> và Lê Xuân Cảnh<sup>3,4</sup>**

**Tóm tắt:** Hai loài Chuột chù răng trắng miền Bắc *Crocidura dracula* và Chuột chù răng trắng miền Nam *C. fuliginosa* có hình thái ngoài và đặc điểm sọ rất giống nhau. Vì vậy, bậc phân loại và hiện trạng phân bố của hai loài này còn nhiều vấn đề gây tranh cãi. Các nghiên cứu định loại dựa trên các bằng chứng về sinh học phân tử trong thời gian gần đây đã thay đổi nhận định về khu vực có sự xuất hiện của hai loài này. Dựa trên những mẫu vật mới được thu thập, nghiên cứu bổ sung hiện trạng phân bố địa lý của hai loài này ở khu vực phía Nam Trung Quốc và Đông Nam Á bằng phương pháp hình thái và sinh học phân tử. Đồng thời, nghiên cứu của nghiên cứu cũng cho thấy sông Mê Kông là ranh giới cách ly địa lý của hai loài trên.

**Từ khóa:** Chuột chù răng trắng, địa sinh học, rào cản, sông Mê Kông.

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