# Full Research Report

บัญชีรายชื่อแมลงน้ำวงศ์ย่อย **Hemerodromiinae (Diptera: Empididae)** ในแหล่งน้ำไหลในเขาหินปูนในระบบนิเวศคาสต์ในประเทศไทย

**Inventory of aquatic Hemerodromiinae (Diptera: Empididae) inhabiting tufa stream environments in tropical karst ecosystems in Thailand**



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**Project completed 08/2563**

**Contract No: DBG6180024**

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This work was generously funded by the Thailand Research Fund (DBG6180024) who are acknowledged with sincere gratitude

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# Inventory of aquatic Hemerodromiinae (Diptera: Empididae) inhabiting tufa stream environments in tropical karst ecosystems in Thailand

**(DBG6180024)**


### Abstract

An inventory was compiled of aquatic dance-flies (Diptera: Empididae) inhabiting tufa and other calcareous waters in Thailand. Communities were dominated by species of *Hemerodromia* Mg. and species of other genera were rare. 5 new species of *Hemerodromia* were described; one reported as new to Thailand and 11 previously known species observed. 6 species had an obligate association with tufa, 2 with calcareous streams, 3 occurred on both tufa and streams and 6 were eurytopic species also found away from karst. The 6 obligate tufa species were absolutely associated with rapid shallow flows of water over tufa at waterfalls but benthic substrate and canopy cover were important environmental variables for most species. Species richness and abundance varied through the monsoon cycle but there were few simple correlations with seasonal changes in water temperature, conductivity and pH. Tufa and its insect communities are severely threatened by human activities (especially tourism & agriculture).

Local and regional-scale endemism was apparent at species-level in *Hemerodromia*. Assemblage similarity in tufa habitats decayed with distance but cluster analysis provided only weak support for community-level endemism at any geographic scale. The hypothesis that cold-adapted aquatic Empididae may have been marooned in cold emergent groundwaters at lower elevations was not supported.

The population genetic structure of an obligate tufa species (*H. conspecta*) with a fragmented distribution across northern Thailand was explored. A median-joining (MJ) haplotype network based on COI sequences indicated a high level of genetic structure and resolved 5 distinct lineages associated with geographic clusters. Haplotype diversity, nucleotide diversity and pairwise comparisons of $F_{ST}$ suggested considerable differentiation among populations but evolutionary distance (using Kimura 2-parameter) was too low to establish any lineage as a discrete species but does provide evidence of ongoing processes of allopatric speciation. Mismatch distribution analysis and low nucleotide diversity associated with high haplotype diversity in three lineages (endemic to Lampang + Chiang Rai, Phrae, Tak + Kanchanaburi) was consistent with the sudden expansion model but very low haplotype diversity of a fourth lineage (Loei) suggested it had historically experienced a profound population bottleneck. Long path lengths in the MJ network suggested prolonged isolation of the Lampang + Chiang Rai, Phrae, Tak + Kanchanaburi and Loei lineages and mismatch distribution analysis indicated that their populations experienced a late Pleistocene expansion (~10,000 – 100,000 bp), entirely consistent with evidence for a climatically driven period of late Cenozoic tufa deposition followed by late Quaternary decline. The allopatric distribution of geographically fragmented and genetically distinct lineages supports the view that vicariant diversification is driving active processes of speciation and microendemism occurring within multiple microrefugia set within a wider matrix of unsuitable habitats.

It is concluded that tropical Tufa ecosystems in Thailand support distinctive, rich and often endemic populations of stenotopic species of considerable biological and conservation interest. Deteriorative changes linked to tourism, water abstraction and agricultural activity may threaten these unique communities.

Incidental observations revealed that silk capture nets of Philopotamidae (Trichoptera) provide accretion sites for calcite deposition and contribute to tufa formation; a rare example of a biomediation in deposition of new rock.



**Keywords**
allopatric speciation, bottleneck, diversity, ecology, endemism, habitat specialism, *Hemerodromia*, limestone, new species, palaeoclimate , refugia, vicariant diversification

**บัญชีรายชื่อแมลงน้ำวงศ์ย่อย Hemerodromiinae (Diptera: Empididae)**
**ในแหล่งน้ำไหลในเขาหินปูนในระบบนิเวศคาสต์ในประเทศไทย**


**บทคัดย่อ**

รวบรวมข้อมูลและจัดทำบัญชีรายชื่อของแมลงน้ำวงศ์ Empididae ที่อาศัยในแหล่งน้ำแบบทูฟา (tufa) และแหล่งน้ำที่มีหินปูนในประเทศไทย ชุมชีวินของแมลงวงศ์นี้ในแหล่งอาศัยที่เป็นหินปูนมีแมลงในสกุล *Hemerodromia* Mg. เป็นสกุลเด่น ขณะที่แมลงในสกุลอื่นๆ พบน้อยมาก การศึกษานี้พบแมลงวงศ์ Empididae ในแหล่งอาศัยที่เป็นหินปูน 16 สปีชีส์ ในจำนวนนี้ 5 สปีชีส์เป็นสปีชีส์ใหม่ของโลก 6 สปีชีส์ พบเฉพาะในแหล่งอาศัยแบบทูฟา 2 สปีชีส์ พบเฉพาะในแหล่งน้ำที่มีหินปูน 3 สปีชีส์พบได้ในแหล่งอาศัยทั้ง 2 แบบ และ 6 สปีชีส์พบในแหล่งอาศัยทั้งที่เป็นหินปูนและแหล่งอาศัยลักษณะอื่น แมลงวงศ์ Empididae จำนวน 6 สปีชีส์ที่พบเฉพาะในแหล่งอาศัยแบบทูฟามีความสัมพันธ์กับลำธารน้ำตื้นที่มีความเร็วของกระแสน้ำสูงในบริเวณน้ำตก อย่างไรก็ตามปัจจัยทางนิเวศวิทยาอื่นๆ ได้แก่ วัสดุพื้นลำธารและการปกคลุมเรือนยอด เป็นปัจจัยสำคัญต่อการปรากฏของหลายสปีชีส์เช่นกัน ความหลากหลายและจำนวนของแต่ละสปีชีส์แปรผันตามฤดูกาลที่สัมพันธ์กับฤดูมรสุมแต่ปัจจัยทางนิเวศวิทยา ได้แก่ อุณหภูมิน้ำ ค่าการนำไฟฟ้าของน้ำ และค่าความเป็นกรด-ด่าง มีความสัมพันธ์น้อยมากกับการเปลี่ยนแปลงของฤดูกาล แหล่งอาศัยทูฟาและแมลงที่พบในแหล่งอาศัยนี้ได้รับผลกระทบรุนแรงจากกิจกรรมของมนุษย์ โดยเฉพาะอย่างยิ่งจากการท่องเที่ยวและการทำเกษตรกรรม

แมลงในสกุล *Hemerodromia* ปรากฏหลักฐานการเป็นสปีชีส์ประจำถิ่นทั้งในระดับท้องถิ่นแหล่งอาศัยและระดับภูมิภาค ความคล้ายคลึงของกลุ่มชีวินในแหล่งอาศัยทูฟาลดลงเมื่อระยะห่างของประชากรเพิ่มขึ้น แต่การวิเคราะห์แบ่งกลุ่มพบความสัมพันธ์เล็กน้อยระหว่างชุมชีวินประจำถิ่นในทุกระยะทางภูมิศาสตร์ ซึ่งไม่สนับสนุนสมมติฐานว่าแมลงวงศ์ Empididae ที่ปรับตัวต่อสภาพอากาศหนาวเย็นอาจมาจากแหล่งอาศัยที่อยู่ในแหล่งน้ำที่มีความเย็นที่ระดับความสูงจากระดับน้ำทะเลไม่มาก

ศึกษาพันธุศาสตร์ประชากรของแมลง *Hemerodromia conspecta* ซึ่งเป็นสปีชีส์ที่จำเพาะกับแหล่งอาศัยแบบทูฟา การวิเคราะห์สายสัมพันธ์ของแฮพโพลไทป์ด้วย median-joining network โดยใช้ลำดับนิวคลีโอไทด์ของยีน COI พบว่าประชากรมีความแตกต่างทางพันธุกรรมสูง โดยแยกเป็นสายวิวัฒนาการที่แตกต่างกัน 5 สายที่สัมพันธ์กับตำแหน่งทางภูมิศาสตร์ ความหลากหลายของแฮพโพลไทป์ ความหลากหลายของนิวคลีโอไทด์ และความแตกต่างทางพันธุกรรมจากค่า $F_{ST}$ บ่งชี้ว่ามีความแตกต่างทางพันธุกรรมระหว่างประชากรสูง แต่ค่าความแตกต่างทางพันธุกรรมนี้ยังไม่เพียงพอที่จะแบ่งแยกสายวิวัฒนาการเหล่านี้เป็นสปีชีส์ที่แตกต่างกันได้ แต่อาจอยู่ในระหว่างกระบวนการแบ่งแยกสปีชีส์แบบอัลโลพาทริก ผลการวิเคราะห์ mismatch distribution ความหลากหลายของนิวคลีโอไทด์ต่ำ แต่ความหลากหลายของแฮพโพลไทป์สูงในสายวิวัฒนาการ 3 สาย ได้แก่ สายวิวัฒนาการลำปางและเชียงราย สายวิวัฒนาการแพร่ และสายวิวัฒนาการตากและกาญจนบุรี มีความสอดคล้องกับปรากฏการณ์การขยายขนาดอย่างรวดเร็วของประชากรในอดีต สำหรับสายวิวัฒนาการเลยที่พบว่ามีความหลากหลายของแฮพโพลไทป์ต่ำเป็นผลจากการลดขนาดของประชากรอย่างรวดเร็วจากปรากฏการณ์คอขวด ซึ่งการขยายขนาดของประชากรอย่างรวดเร็วนั้นเกิดขึ้นในช่วงท้ายของยุคไพลสโทซีนประมาณ 10,000 – 100,000 ปี ที่ผ่านมา สอดคล้องกับหลักฐานทางธรณีวิทยาที่บ่งชี้ว่าเกิดการเปลี่ยนแปลงสภาพภูมิอากาศที่ส่งเสริมการเกิดทูฟาในยุคซีโนโซอิกก่อนที่จะลดลงในยุคปัจจุบัน การกระจายทางภูมิศาสตร์ของประชากรที่แบ่งแยกทางภูมิศาสตร์และความแตกต่างทางพันธุกรรมอย่างชัดเจนของแต่ละสายวิวัฒนาการสนับสนุนแนวคิดเรื่องการแบ่งแยกของประชากรทางภูมิศาสตร์ส่งเสริมให้เกิดการแตกแขนงของสปีชีส์และประชากรประจำถิ่นภายในพื้นที่ที่มีแหล่งอาศัยย่อยที่ถูกแบ่งแยกด้วยแหล่งอาศัยที่ไม่เหมาะสมต่อการดำรงชีพของสปีชีส์

ผลการศึกษานี้สรุปได้ว่าระบบนิเวศทูฟาในเขตร้อนชื้นในประเทศไทยส่งเสริมการแบ่งแยกของประชากรที่มีความหลากหลายและเฉพาะถิ่นของสปีชีส์ที่มีความต้องการปัจจัยทางนิเวศวิทยาที่จำเพาะ ซึ่งมีความสำคัญต่อการศึกษาและการอนุรักษ์ การเสื่อมสภาพของแหล่งอาศัยเหล่านี้ที่เชื่อมโยงกับการท่องเที่ยว และกิจกรรมทางการเกษตรอาจส่งผลกระทบต่อแหล่งอาศัยที่มีเอกลักษณ์นี้

การค้นพบโดยบังเอิญในการศึกษานี้พบว่าใยไหมของแมลงในวงศ์ Philopotamidae (Trichoptera) เป็นแหล่งสะสมของหินปูนที่เกี่ยวข้องกับการสร้างทูฟา ซึ่งเป็นตัวอย่างที่พบน้อยมากของการกำเนิดหินใหม่

**คำสำคัญ:** การแตกแขนงของสปีชีส์แบบอัลโลพาทริก, ปรากฏการณ์คอขวด, ความหลากหลาย, นิเวศวิทยา, ประจำถิ่น, ความจำเพาะกับแหล่งอาศัย, Hemerodromia, หินปูน, สปีชีส์ใหม่, ภูมิอากาศบรรพกาล, แหล่งอาศัยจำเพาะ, การแตกแขนงจากการแบ่งแยกทางภูมิศาสตร์

# 1. INTRODUCTION

Limestone karst landforms in southeast Asia are composed primarily of Ordovician, Permian Carboniferous and some Silurian and Jurassic marine carbonate deposits that have been uplifted by tectonic movements (Stait & Burrett, 1984; Pfeffer, 2013) and now form numerous isolated, limestone blocks that cover about 10% of total land area in the region (Day & Urich, 2000). The highly specialized habitats present in rugged karst landscapes have been shaped by hydrological dissolution of carbonates in the bedrock resulting in shallow alkaline soils enriched with Ca, Mg and K. The thin soils and exposed rock surfaces that experience rapidly changing extremes of water availability, temperature, humidity, evaporation rates, insolation and exposure to wind, provide challenging environments that are exploited largely by highly specialized organisms such as calciphiles and xerophiles (Sterling *et al*., 2006; Bystriakova *et al*., 2019). A specialized troglobiont fauna inhabits the subterranean groundwater aquifer that is usually present beneath karst landforms (Zakšek *et al*., 2009). Individual blocks of karst habitat effectively form habitat 'islands' geographically isolated from each other by extensive areas of habitat unsuitable for the highly specialized karst biota, and which constrains geographical ranges of species, spatially restricts gene-flow between populations and promotes high levels of local endemism in terrestrial, surface water and groundwater biota (Zakšek *et al*., 2009; Bilandžija *et al*., 2013; Ivković & Plant, 2015; Gao *et al*., 2015). As such, karst islands provide natural laboratories for investigating mechanisms of divergence and speciation.

**Consequences of genetic isolation of species with discontinuous distribution**

Species that require specialist, discontinuous, fragmented or patchily distributed habitats generally show high levels of population genetic structure (Verovnik *et al*. 2004; Klobučar *et al*. 2013; Pramual & Wongpakam 2013; Pramual & Pangjanda 2015; Mamos *et al*. 2014). This may be especially true for aquatic insects in which population structure is intimately linked to historical hydrogeological fragmentation patterns (Previšić *et al*. 2009; 2014). In fragmented habitats, this genetic structure arises when there is limited gene flow amongst populations of species with low dispersal abilities, and results in population differentiation through geographic isolation (Slatkin 1993; Brändle *et al*. 2007; Johannesen *et al*. 2010). Furthermore, historical vicariant events associated with habitat fragmentation dividing matrix populations of species with low vagility often lead to allopatric speciation, can influence geographical range (Beebee & Rowe 2004), cause disjunct distributions (Runemark *et al*. 2012; Ivković & Plant 2015) and because stenotopic species adapted to specialist habitats show strong niche conservatism and tend to track their ancestral habitats, may become 'marooned' in fragmented palaeorefugia (Nekola 1999; Chung *et al*. 2014).

Unfortunately karst ecosystems are fragile and are severely threatened throughout Thailand and Southeast Asia by many anthropic influences such as mining of limestone for cement, damage to the hydrology through abstraction or hydroelectric dams, agricultural encroachment and tourism (Clements *et al*., 2006; Latinne, 2011; Hughs, 2017). Extinction risk is high in species with restricted ranges (Purvis et al., 2000; Harvey, 2002) and the low dispersal ability and isolation of karst species renders them inherently vulnerable to environmental changes and they may be particularly at risk from climate change (Anderson & Ferree, 2010; Wang *et al*., 2019).

The calcareous groundwater resurging from karst aquifers into downstream springs, seeps, streams and rivers supports rich assemblages of calciphile aquatic insects, at least in temperate or Mediterranean zones were they have been investigated (Previšić *et al*. 2007; Mori & Brancelj, 2006; Gerecke *et al*. 2011). In places where groundwater at ambient temperatures is supersaturated with carbonates derived from solution weathering of the limestone, deposits of tufa (calcite, solid $CaCO_3$) may form, often developing into substantial features such as cliffs and terraces over which water flows. Tufa supports little-known but unique and highly stenotopic communities of aquatic insects such as Plecoptera (Ridl, 2018), Trichoptera (Šemnički *et al* 2012) and Diptera (Ivković *et al*., 2010; Kvifte & Ivković, 2018).

**Tufa**

Tufa is unlithified secondary deposits of calcite (crystalline calcium carbonate, $CaCO_3$) formed at ambient temperatures in riverine or lacustrine waters. Tufa formations are widespread globally but are most frequent about springs and upper water courses in tropical and subtropical karst areas where carbonates produced by solution weathering are reworked through the karst and eventually deposited as tufa. Outgassing of $CO_2$ from supersaturated $CaCO_3$ solution leads to the precipitation of calcite. Although the reaction is ostensibly simple ($Ca^{2+} + 2HCO_3 \rightarrow CaCO_3 + CO_2$), the outcome is complicated by a 3-phase equilibrium between gas ($CO_2$), solid ($CaCO_3$) and aqueous solutions of $Ca^{2+}$, $HCO_3^-$ and $CaCO_3$. Calcite precipitation is also influenced by factors such as water flow rate and the air-water interface area (Zhang *et al*. 2001) and may be mediated by biotic factors that provide nucleation or entrapment sites for calcite (Martín-Algarra *et al*. 2003; Kepčija et al. 2006; Pedley *et al*. 2009; Contemporary insect communities of actively growing “living tufa” thus provide an interesting example of biotic involvement in ongoing geological processes (for example this study found silk capture nets of philopotomid Trichoptera were contributing to tufa accretion processes (Plant, 2019) Karst habitats are shaped mostly by erosive processes with net removal of rock due to hydrological dissolution by meteoric water, with carbonates weathered from the rock being hydrologically transported away from the karst (in calcareous streams and rivers). In contrast tufa represents a depositional zone. It is an inherently transient habitat, influenced by dynamic fluvial processes. Active tufa is a rare, highly fragmented and diminishing habitat viewed at evolutionary, spatial and temporal scales (Goudie *et al*.1993; Zhang *et al*. 2001; Fubelli *et al*. 2013; Glover & Robertson 2003).

Southeast Asian karsts have a rich endemic fauna of plants and both vertebrate and invertebrate animals (Latinne *et al*., 2011) and although still little known, are clearly of great biological importance, often forming biodiversity ‘hotspots’ (Clements *et al*., 2006). However, the fauna of calcareous surface waters of Southeast Asian karst, especially that inhabiting tufa, is almost completely unstudied. Pramual & Pangjanda (2015) have reported specialist calciphile Simuliidae (Diptera) and Plant (2015) previously described three species of the aquatic fly genus *Hemerodromia* Mg. (Empididae subfamily Hemerodromiinae) that were apparently associated with active tufa systems in Thailand. Species of Hemerodromiinae (and the related aquatic empidid subfamily Clinocerinae) are known elsewhere from tufa systems (Ivković *et al*., 2010, 2015; Ivković & Plant, 2015). Adults and larvae of both subfamilies are predatory and make significant contributions as secondary consumers in various spring and stream microenvironments (Ivković *et al*., 2007, 2012).

The biological and conservation significance of active tufa systems and other calcareous waters is largely unrecognized in Thailand where the lack of taxonomic studies makes it impossible to identify the species that are present and it is consequently not possible to rationally appraise species richness, endemism or geographical ranges. We need first to

describe the species, then chart their distributions at the landscape level and understand the specific biotopes where they occur (e.g. springhead, tufa cascades and barriers, downstream streams and rivers). Only then will we be able to study their ecology and population genetics prioritize habitats and communities or areas of conservation importance.
As noted earlier, aquatic Empididae in the subfamilies Hemerodromiinae and Clinocerinae include many stenotopic calciphiles and at least three Thailand species apparently have

**Aquatic Empididae**

- Empididae comprises a globally widespread and often abundant group of Diptera (flies). Thailand has ~150 known species but >1,000 remain undesctibed.
- Two subfamilies of Empididae have aquatic immature stages.-

  **Cinocerinae** (genera occurring in Thailand = *Clinocera, Dolichocephala*)
  **Hemerodromiinae** (genera occurring in Thailand = *Hemerodromia, Achelipoda, Anaclastoctedon, Chelipoda* and *Chelifera*)

- Adults of all Empididae are predators of other insects and those of Hemerodromiinae have raptorial limbs used for seizing prey
- Modern taxonomic revisions are available for most of these genera (Sinclair & Plant, 2017; Plant, 2009, 2009a, 2010, 2015).

associations with tufa. Improved knowledge of their wider taxonomy, biogeography and ecology in Thailand (Plant, 2009, 2009a, 2010, 2015; Plant *et al.*, 2011, 2012, 2019; Sinclair & Plant, 2017) renders their calciphile communities tractable to study. This project sought to advance knowledge of calciphile Diptera in Thailand by: -

(1) Compile an Inventory of aquatic Empididae associated with calcareous waters throughout Thailand.
(2) Describe new taxa.
(3) Identify the extent of endemism at species and community level.
(4) Assess population genetic structure and genetic isolation.
(5) Test the hypothesis that climatically relict cold-adapted aquatic Empididae may have been marooned in cold emergent groundwaters at lower elevations.
(6) Evaluate the influence of environmental conditions (pH, conductivity, benthos, canopy cover etc.) on structure and composition of assemblages.
(7) Identify threats to assemblages

# 2. METHODS

## 2.1 *Study sites*

Study sites were 73 streams, rivers, springs and tufa systems (mostly barrier streams and cones ≤2 km downstream of their resurgent sources) on or originating on karst limestone landforms (Table 1, Fig 12B). More sites were examined but not sampled due to access problems or destructive anthropic influences (for example many springheads have pump-houses built over them or have long ago been incorporated into Buddhist temples). Samples were taken on various dates between October 2019 and March 2020. All major karst areas of Thailand were sampled:- (1) The Lower Northeast Region in Loei and Phitsanulok and on the western margins of the Isaan Plateau in Chayaphum, Lopburi, Saraburi and Nakhon Ratchasima provinces composed of Carboniferous-Permian limestones of the Saraburi Group (2) The Northern Region with Carboniferous-Permian limestones mostly of the Ngao Group, Doi Chiang Dao or Phawar formations in Chiang Mai, Chiang Rai, Mae Hong Son, Lampang, Phrae and Nan provinces (3) The Western Region in Kanchanaburi and Tak provinces with a surface geology of Ordovician (Thung Song Group), Permian (Ratburi and Ngao groups) and some Jurassic limestones (4) The Southern Region in Phangnga, Surat Thani, Krabi, Trang, Phatthalung and Satun provinces with Ordovician (Thung Song Group) Permian (Ratburi and Ngao groups) and various Carboniferous-Permian formations.

## 2.2 *Taxonomy and Identification*

Higher taxonomy followed hemerodromiine phylogeny of Plant (2011). Adult Hemerodromiinae were determined using Plant (2009a, 2009b, 2015) or Yang & Yang (2004) and new species were described (see later section and Plant, 2020). Clinocerinae were represented in the samples only by *Clinocera* Mg. sp that remains undescribed. Reference material is housed at Queen Sirikit Botanical Garden (Entomology Section), Mae Rim, Chiang Mai, Thailand and type depositories of new species are indicated in Plant (2020). Species determinations were based on morphological characters of proven value (in many cases accurate determination was only possible by examination and dissection of male terminalia; many females were impossible to determine) Descriptions of new species used the same characters of proven value with particular emphasis on taxonomically and phylogenetically important characters of the male terminalia considered as apomorphic within *Hemerodromia*.

## 2.3 *Analysis of environmental influences*

Adult Empididae were collected from marginal, overhanging and emergent vegetation using a sweep net. At each locality, the precise sample location data was recorded as:- (a) ≤1 m of ‘fast films’ [shallow rapid water flows on tufa barriers, bosses and cones] (b) ≤25 m of waterfalls, and (c) at locations remote from fast films or waterfalls. Occurrence of tufa, electrical conductivity, pH, temperature and elevation, were recorded and mean water depth and flow rate were determined from ≥10 replicate measurements. Canopy coverage (%) and percentage composition in the benthos of rock (or more usually, tufa accretion on solid surfaces), cobbles, sand, gravel and silt were estimated. The presence and extent of any anthropic influences on sites was noted.

Mean, SE, ANOVA, Kruskal-Wallis tests, ordination (using canonical correspondence analysis (CCA) and with non-metric multi-dimensional scaling (MDS) applying Bray-Curtis similarity), cluster analysis (CA) using unweighted pair-group average and Jaccard similarity) and calculation of distance between geographical sites were performed in PAST 3.02a or 2.17b (Hammer *et al*., 2001). Continuously variable environmental parameters were $\log_{10}$ transformed for ordination.

For tufa-inhabiting species the proportion of all individuals of each species found on tufa ($P_t$), in close association with fast films ($P_{ff}$) and near waterfalls ($P_w$) was calculated

### 2.4 *DNA extraction, polymerase chain reaction (PCR) amplification and DNA sequencing*

DNA was extracted from single, whole specimens of adult *insects* using GF-1 Tissue DNA extraction kit (Vivantis, Selanggor Darul Ehsan, Malaysia). DNA fragments (655 bp) of the cytochrome c oxidase subunit I (COI) gene were amplified using the primers LCO1490 (5′-GGTCAACAAATCATAAAGATATTGG-3′) and HCO2198 (5′-TAAACTTCAG GGTGACCAAAAAATCA-3′) (Folmer *et al*. 1994). Polymerase chain reactions (PCR) for specimens of *Hemerodromia* was performed in a total volume of 50 ml containing 2 ml of DNA template, 2 ml of each primer (10 mmol/L), 3 ml of 50 mmol/L $MgCl_2$, 5 ml of 10x PCR buffer, 1.6 ml of 10 mmol/L dNTPs, 0.4 ml of Taq DNA polymerase (5 U/ml) (Vivantis, Malaysia) and 34 ml sterile $ddH_2O$. Temperature profile was as follows: 5 cycles of 94°C for 2 min followed by 5 cycles of 94°C for 30 sec, 45 °C for 40 sec, and 72 °C for 1 min, followed by 35 cycles of 94 °C for 30 sec, 51 °C for 40 sec, and 72 °C 1 min, with final extension at 72 °C for 10 min. PCR products were checked using 1% agarose gel electrophoresis and purified using HiYield Gel/PCR DNA Extraction Kit (RBC Bioscience, Taiwan) in accordance with the manufacturer's instructions. Purified PCR products were sent for sequencing at 1st BASE sequencing service (Malaysia) using the same primers as in the PCR.

### 2.5 *Analysis of population genetics*

Sequences were aligned using BioEdit version 7.0.5.3 (Hall 1999) with final manual adjustment. Relationships between haplotypes were estimated using the median joining (MJ) network (Bandelt *et al*. 1999). The MJ network analysis was performed in network, version 10.0 (http://www.fluxus-engineering.com) based on 655 bp sequences obtained in the present study. Haplotype diversity (h) and nucleotide diversity ($\pi$) were calculated in Arlequin version 3.5.1.2 (Excoffier & Lischer 2010). Population pairwise $F_{ST}$ calculated in Arlequin based on Kimura 2-parameter was used to infer the genetic structure. The significance test statistic was obtained from 1023 permutations. To avoid bias as a result of a small sampling size, populations with sample sizes of less than three were not included in the $F_{ST}$ analysis. The Mantel test (Mantel 1967) was used to examine the relationship between genetic distance ($F_{ST}$ from Arlequin) and geographical distance (km) to test an isolation-by-distance model. The Mantel test was performed in IBD, version 1.53 (Bohonak 2002) using 1000 randomizations.

The mismatch distribution analysis also calculated in Arlequin, was used to infer demographic history of the *H. conspecta*. The mismatch distribution graph is expected to be unimodal in a population that has undergone recent demographic expansion (Roger & Harpending 1992). Deviation from the sudden expansion model was tested using the sum-of-square deviation (SSD) and Harpending's raggedness index (Harpending 1994). Tests of significance for SSD and Harpending's raggedness index were based on bootstrapping with 1,000 replications. If the mismatch graph was fitted to the sudden population expansion model, population expansion time was calculated using $\tau = 2ut$ where $u = m_T\mu$, $m_T$ is the number of nucleotides of the sequences under study and μ is the mutation rate per nucleotide (Roger & Harpending 1992) and assuming 1 and 12 generations per year (see discussion) for *H. conspecta* and a divergence rate of 2.3% per 1000000 years for insect mitochondrial DNA (Brower 1994). Because population subdivision can affect mismatch distributions (Marjoram & Donnelly, 1994) they were analysed separately for each lineage identified from the MJ network.

**3. *Taxon abbreviations***

*Akan*, *Achelipoda kanaklua* Plant; *Akha, Achelipoda khakema* Plant; *Cnak, Chelipoda nakropa* Plant; *Hacu, Hemerodromia acutata* Grootaert, Yang & Saigusa; *Hali, Hemerodromia aliaextricata* Plant, *Hani, Hemerodromia anisoserrata* Plant; *Hano, Hemerodromia anomala* Plant; *Hcon, Hemerodromia conspecta* Plant; *Hdem, Hemerodromia demissa* Plant; *Hdep, Hemerodromia deprimatura* Plant,; *Hfla, Hemerodromia flaviventris* Yang & Yang; *Hfur, Hemerodromia furcata* Grootaert, Yang & Saigusa; *Hfus, Hemerodromia fusca* Yang & Yang; *Hmen, Hemerodromia menghaiensis* Yang & Yang; *Hnam, Hemerodromia namtokhinpoon* Plant; *Hore, Hemerodromia oreotenebraea* Plant; *Hpai, Hemerodromia pairoti* Plant; *Hsam, Hemerodromia samoha* Plant; *Hyun, Hemerodromia yunnanensis* Yang & Yang; *Hzet, Hemerodromia zetalutea* Plant.

# 4. RESULTS

## 4.1 Taxonomy

### 4.1.1 Inventory of Taxa Reported

1888 adult Empididae were collected. *Hemerodromia* was the dominant genus (99.5% of individuals, n=1880) of which 1695 specimens were determined or newly described as representing 17 species. 205 specimens remained unidentifiable (females not associated with males, damaged or teneral males, putative new species etc.). Two specimens of an undescribed aquatic *Clinocera* Mg. sp. (Clinocerinae) and 6 individuals of three species (*Akan*, *Akha* and *Cnak*) in the semi-aquatic or possibly terrestrial hemerodromiine tribe Chelipodini were excluded from further analysis which focused only on the strictly aquatic genus *Hemerodromia*.

### 4.1.2 SYSTEMATIC LIST

**Empididae: Hemerodromiinae: Hemerodromiini**

***Hemerodromia aliaextriata*** Plant, 2015
[Zootaxa (2020) 5758, 551 (Figs 1–2, 12)] +*NEW SPECIES*+
***Hemerodromia deprimatura*** Plant, 2015
[Zootaxa (2020) 5758, 552 (Figs 3–4, 13)] +*NEW SPECIES*+
***Hemerodromia oretenebraea*** Plant, 2015
[Zootaxa (2020) 5758, 553 (Figs 5–7, 14)] +*NEW SPECIES*+
***Hemerodromia pairoti*** Plant, 2015
[Zootaxa (2020) 5758, 555 (Figs 8–9, 15)] +*NEW SPECIES*+
***Hemerodromia samoha*** Plant, 2015
[Zootaxa (2020) 5758, 556 (Figs 10–11, 16)] +*NEW SPECIES*+
***Hemerodromia acutata*** Grootaert, Yang & Saigusa, 2000
***Hemerodromia anisoserrata*** Plant, 2015
***Hemerodromia anomala*** Plant, 2015
***Hemerodromia conspecta*** Plant, 2015
***Hemerodromia demissa*** Plant, 2025
***Hemerodromia flaviventris*** Yang & Yang, 1991
***Hemerodromia furcata*** Grootaert, Yang & Saigusa, 2000
***Hemerodromia fusca*** Yang & Yang, 1986
***Hemerodromia menghaiensis*** Yang & Yang, 1988 +*NEW TO THAILAND*+
***Hemerodromia namtokhinpoon*** Plant, 2015
***Hemerodromia yunnanensis*** Yang & Yang, 1986
***Hemerodromia zetalutea*** Plant, 2020.

**Empididae: Hemerodromiinae: Chelipodini**

*Achelipoda kanaklua* Plant, 2009
*Achelipoda khakema* Plant, 2009
*Chelipoda nakropa* Plant, 2009

**Empididae: Clinocerinae**

*Clinocera* Mg. sp. indet

### 4.1.3 Descriptions of New Species of *Hemerodromia*

**Type Depositories** All type material is deposited with Entomology Section, Queen Sirikit Botanic Garden, Mae Rim, Chiang Mai (QSBG)

*Systematic Statement*
*Hemerodromia* Meigen, 1822: 61. Type species: *Tachydromia oratoria* Fallén, 1815, des. Rondani, 1856: 148. [I.C.Z.N., opinion 2347, Case 3589 (2014) conserved usage of *Hemerodromia* Meigen, 1822 by setting aside all type species fixations for *Hemerodromia* Meigen, 1822 prior to that of *Tachydromia oratoria* Fallén, 1815, by Rondani, 1856].

***Hemerodromia aliaexstriata*** (Figs 1–2) [sp. nov in Plant, 2020]
**Diagnosis**. A predominantly orange yellow species with a dark median stripe on the scutum becoming wider posteriorly, a yellow scutellum and with dark marks between the front coxae and on the katepisternum. The male cercus is broad in lateral view with bluntly pointed dorsoapical and ventroapical processes and the epandrium is narrow and elongate, extending beyond the tip of the cercus.
**Description**. Male: body length 2.6 mm; wing length 2.0 mm. Head. Rather elongate, ~1.5X long as wide; upper occiput slightly bulging, making smooth angle where meeting vertex. Black with paler dusting. Antenna and mouthparts whitish. One pair of minute reclinate ocellar setulae; 3–4 pairs of minute vertical setulae only about as long as ocellars. Antenna with postpedicel ~1.8–2.0X long as wide, stylus somewhat shorter. Thorax. Not quadrate anteriorly; anterior margin of scutum gradually curving in lateral view. Ground colour orange yellow; scutum with median brownish black stripe, narrow anteriorly, becoming as wide as base of scutellum posteriorly; mediotergite broadly darkened medially; strong black mark between insertion points of front coxae and another smaller oblique mark on katepisternum; small dark marks also present immediately in front of base of wing, obliquely about prothoracic spiracle and on sutures of laterotergite. All setae whitish yellow, minute; notopleural setulae present but very small, scutellars apparently absent. Legs. Yellowish, distal tarsal segments somewhat darkened. C1~1.1X long as distance between base of C1 and C2. F1 hardly longer than C1, ~4.0X long as wide, strongly and evenly inflated, slightly constricted on proximal 0.2; femoral formula ~ 6–8/22–25/21–24/5–6, denticles black, rows converging apically; spines yellow except 1–2 near base black. T1 ~ 0.7X long as F1; evenly curved, ventral face shallowly concave, with 2 rows of ~20 sharply pointed spinose setae ventrally; ventroapical spur weakly developed; distinct ventroapical erect black spinose seta present. Mid and hind legs slender with only small setulae. Wing. Membrane darkened by greyish microtrichia on distal 0.9, paler about base of wing. Veins greyish black, R4+5 darker still. Veins C and R1 yellowish at extreme base. Marginal setulae dark. R2+3 almost linear, joining C ~0.8–0.9X distance between ends of R1 and R4. Length of C between ends of R2+3 and R4 about as long as R4. R4 slightly S-shaped. R5 ~ 2.0X long as R4, almost linear. R4+5 fork distal to M1+2 fork by ~ 1.3X length of R4. M1 almost linear, slightly convergent with R5, becoming parallel at extreme tip. Cell bm+dm short, ending at tip of R1. Halter yellowish white. Abdomen. Black dorsally, paler ventrally, tergites 1 and 2 yellowish. Terminalia. Black with dark setae. Cercus (Figs 1–2) short, not extending beyond tip of epandrium; rather broad in lateral view with short bluntly pointed dorsoapical process and longer ventroapical process; outer surface with fine black hairs.

Epandrium long and narrow with fine black hairs (Fig. 1). Hypandrium small, strongly arched, bare. Phallus with pair of apical hook-like processes. Female: similar to male but scutum with median stripe somewhat narrower anteriorly and abdomen yellowish ventrally. Tip of abdomen truncate, terminalia rather short, hardly extensible and not at all ovipositor-like.

**Type material**. **HOLOTYPE** ♂: **THAILAND**, Mae Hong Son Province, river near Susa waterfall, 306 m, 19.47797°N, 98.12089°E, 3.iv.2019, netted A.R. Plant (QSBG). PARATYPES: 2♀, same data as holotype (QSBG); 1♂, 3♀, Mae Hong Son Province, Susa waterfall, tufa cliff at confluence of tufa str. and river, 305 m, 19.47457°N, 98.12421°E, 3.iv.2019, netted A.R. Plant (QSBG).

**Etymology**. The specific epithet is a concatenation of the Latin alia ex meaning 'different from' and striata in reference to this species not being identical with *H. striata* Yang & Yang. It is used as a noun in apposition.

**Remarks**. The markings on the scutum of this species somewhat resemble those of *H. acutata* Grootaert, Yang & Saigusa (in which the width and intensity of the median dark stripe varies greatly) or even *H. systoechon* Plant (in which the dark scutal marking is usually very broad with diffuse edges) from which it may readily be separated by the male terminalia. A generally similarly broad cercus with bluntly pointed dorsoapical and ventroapical processes suggests close affinities with two extralimital species, *H. oratoria* (Fallén) (Holarctic distribution), and *H. striata* Yang & Yang from the eastern Palaearctic (China, Beijing). The latter species also has an elongate epandrium. *Hemerodromia aliaexstriata* sp. nov. is known from a calcareous river in the extreme northwest of Thailand.

### ***Hemerodromia deprimatura*** (Figs 3–4) [sp. nov. in Plant, 2020]

**Diagnosis**. A dirty yellow species with a triangular brownish marking behind eye on lower occiput. Male terminalia with inner margin of cercus evenly concave in dorsal view, tip of surstylus L-shaped and a narrow process emerging beyond tip of surstylus (possibly lower surstylus) with very narrow shaft and small black subcircular process apically. Description. Male: body length 2.1 mm; wing length 1.7 mm. Head. Slightly elongate. 1.4X long as deep, upper occiput making smooth curve where meeting vertex, hardly bulging. Dirty yellow with pale dusting, lower occiput with brown mark behind eye becoming broader ventrally (appearing triangular in lateral view). Antenna and mouthparts whitish. One pair of minute reclinate ocellar setulae; 3–4 pairs of vertical setulae positioned in linear series parallel with upper eye margin, clearly distinguished from other fine hairs; postocular and lower occipital setulae minute. Antenna with postpedicel ~2.5X long as wide, stylus of similar length. Thorax. Dirty whitish yellow with greyish dust; darker on posterior margins of scutellum and mediotergite; blackish about anterior spiracle, 'scutoscutellar eye' and ventrally between insertion points of front coxa. All setae yellowish, minute, but notopleural distinct and pair of weak scutellars. Legs. Dirty whitish yellow, apical tarsomeres of all legs very slightly darker. C1 ~1.2–1.3X longer than distance between base of C1 and C2; C1, C2 and C3 with all setulae minute. F1 ~1.0–1.1X long as C1; F1 4–5X long as wide, very slightly constricted on inner face at 0.2 from base. F1 with two parallel rows of 17–21 minute black denticles ventrally, either side of which is linear series of less numerous and inconspicuous fine yellow bristles, noticeably stronger around 0.2 from base. T1 ~0.8X long as F1, slightly curved, ventral face shallowly concave bearing two rows of minute, sharply pointed, blackish spinose setulae; ventroapical spur weakly developed; distinct ventroapical erect black spinose seta present. Wing. Membrane clear but darkened on distal 0.9 by blackish microtrichia. Veins greyish on distal 0.9, paler on proximal 0.1. R2+3 linear, joining C ~ 0.7X distance between ends of R1 and R4. Length of C between ends of R2+3 and R4 ~1.7–1.8X long as R4. R4 linear, not at all Sshaped, angle with R5 at extreme base ~ 70°. R5 ~2.2X long as R4. R4+5 fork distal to M1+2 fork by ~1.5X length of R4. R5 and M1 almost linear, distinctly convergent distally. Cell bm+dm rather long, ending just beyond end of R1. Halter dirty white. Abdomen. Tergites blackish but tergite 1 pale with narrow transverse black fascia at middle, tergite 7 and all sternites more uniformly pale. All setulae small and dark, longer on posterior margin of 7th segment. Terminalia dirty yellowish. Cercus (Figs 3–4) elongate, extending slightly beyond tip of epandrium, slightly inflated apically in lateral view, some fine hairs dorsally and on inner face slightly stronger dorsoapically; inner margin distinctly evenly concave in dorsal view. Epandrium (Fig. 4) bluntly pointed apically, covered with fine tomentum and a few strong bristles apically. Surstylus present, extending distinctly beyond tip of epandrium, rather broad, L-shaped apically with broad dorsal process; narrow process emerging beyond tip of surstylus (possibly lower lobe of surstylus) with very narrow shaft and small black subcircular process apically (apparently flat on inner face, more convex on outer). Hypandrium (Fig. 4) large, pointed apically, covered with fine tomentum and only a few distinct bristles. Female: similar to male but abdomen with terminalia elongate, somewhat ovipositor-like.

**Type material**. **HOLOTYPE** ♂: **THAILAND**, Phang Nga Province, Sa Nang Namora waterfall, tufa stream, 59 m, 8.51063°N, 98.54140°E, 16.i.2019, netted A.R. Plant (QSBG). PARATYPES: 2♂, 2♀, same data as holotype (QSBG).

**Etymology**. The specific epithet is derived from the Latin deprimatur, meaning 'perfectly poised' in reference to the apparent hunting posture adopted by adult *Hemerodromia* when searching for prey.

**Remarks**. This species is very similar to and clearly closely related to *H. anisoserrata* Plant and H. *ocellata* Plant from Thailand and *H. serrata* Saigusa and Yang from China (Henan). The new species may be distinguished by the triangular brown marking on the lower occiput and the form of the male terminalia in which the cercus is evenly curved on the inner face (irregular in *anisoserrata* / *ocellata* / *serrata*), the elongate bluntly pointed epandrium (apically broad in *anisoserrata*, short and broad in ocellata, apically narrower in serrata) and the complex shapes of the two elongate processes of the upper and lower surstylus (very different shapes in *anisoserrata* / *ocellata* / *serrata*). *Hemerodromia deprimatura* sp. nov. is only known from a calcareous tufa stream in southern Thailand.

### ***Hemerodromia oretenebraea*** sp. nov. (Figs 5–7) [sp. nov. in Plant, 2020]

**Diagnosis**. A blackish species with head and thorax rather quadrate. The anterior of the wing is covered with dark microtrichia, usually contrasting strongly with the posterior part. The front femur is deep black, contrasting strikingly with otherwise pale yellowish legs. Description. Male: body length 2.8 mm; wing length 1.8 mm. Head. Rather short, only slightly longer than deep, rather quadrate (especially in dorsal view); upper occiput vertical in lateral view, upper part slightly but distinctly bulging where meeting vertex. Black, dusted greyish; antenna blackish; mouthparts pale yellow, proboscis with darker tip. One pair reclinate ocellar setulae; 4–5 pairs small indistinct vertical setulae, about as strong as ocellars; pair of minute frontals behind base of antenna. Antenna with postpedicel noticeable stronger and more bristle-like about 0.2 from base.T1 ~ 0.7X long as F1, sublinear, ventral face only slightly concave; with one row of ~12 sharply pointed spinose setae ventrally; ventroapical spur weakly developed; distinct ventroapical erect black spinose seta present. Mid and hind legs slender with only small setulae. Wing. Membrane distinctly darkened with blackish microtrichia, especially anteriorly of M in distal part of wing beyond radial fork; cell bm+dm, base and posterior part of wing paler. Veins greyish black, R2+3, R4+5, R4 and R5 distinctly darker and somewhat thickened. Marginal setulae greyish black. R2+3 linear, joining C ~ 0.7–0.8X distance between ends of R1 and R4 . Length of C between ends of R2+3 and R4 ~ 1.0–1.1X long as R4. R4 slightly S-shaped, angle with R5 at extreme base ~ 65–70°. R5 ~ 1.7–2.0X long as R4, almost linear, almost parallel with M1 but divergent apically. Cell bm+dm rather long, ending distinctly beyond end of R1. Halter greyish white. Abdomen. Blackish, all setulae inconspicuous, very short, dark, a few longer setulae on sternites 7 and 8. Terminalia. Black with dark setae. Moderately small and compact. Cercus (Figs 5–6) elongate, extending beyond tip of epandrium, narrow in lateral view, inner margin distinctly evenly concave in dorsal view. Epandrium (Fig. 5) rather ovate with some fine setae and patch of microscopic pile distally. Surstylus apparently more or less fused with tip of epandrium, bearing two distinct spines (Fig. 7). Hypandrium (Fig. 5) short, compact, distinctly arched. Apex of phallus bifid in posterior view. Female: similar to male but abdomen with shorter setulae and terminalia elongate, ovipositor-like.

**Type material**. **HOLOTYPE** ♂: **THAILAND**, Loei Province, Hin Pha Ngam, Pang Din waterfall, tufa stream, 628 m, 17.06639°N, 101.74813°E, 5.iii.2019, netted A.R. Plant (QSBG). PARATYPES:

4♀ same data as holotype (QSBG); 1♀, Phang Nga Province, Sa Nang Namora waterfall, tufa stream, 59 m, 8.51063°N, 98.54140°E, 16.i.2019, netted A.R. Plant (QSBG); 7♂, Lampang Province, Wang Kaew waterfall, 568 m, 19.3164°N, 99.6628°E, 14.i.2020, netted A.R. Plant (QSBG); 1♂, Phrae Province, Huai Rong tufa waterfall, 450 m, 18.4424°N, 100.4495°E, 18.i.2020, netted A.R. Plant (QSBG). Additional material. 2♂, 1♀, Chiangrai Province, Doi Luang National Park, Pu Kaeng waterfall, 540 m, 19.4422°N, 99.6949°E, 15.i.2020, netted A.R. Plant (QSBG); 2♂, 4♀, Lampang Province, Mae Kae waterfall, 537 m, 18.7457°N, 99.8164°E, 16.i.2020, netted A.R. Plant (QSBG).
**Etymology**. The specific epithet is derived from the Latin ore, meaning 'edge' and tenebrae meaning 'darkness' in reference to the darker membrane on the anterior part of the wing.
**Remarks**. The quadrate, head and thorax and relatively simple and small male terminalia with fused surstylus bearing two spines suggest that this species is related to *H. conspecta* Plant, also from Thailand. However, it may readily be distinguished by the bulging occiput, vein C being all dark (basally yellow in *H. conspecta*), wing distinctly darkened anteriorly and having F1 black (yellow in *H. conspecta*) contrasting strongly with the rest of the legs as well as shape of the cercus and surstylus of the male terminalia. In some examples the wing is more generally darkened although still darker anteriorly. Although darkened front femora are found in several other species of *Hemerodromia*, the front coxa is seldom clear yellow as in the new species where the contrast between the front femur and coxa is striking, and is a rare example of leg colour being diagnostically useful in the genus. *Hemerodromia oretenebraea* sp. nov. has a disjunct distribution occurring at several sites in the north and one in the south of the country.

***Hemerodromia pairoti*** sp. nov. (Figs 8–9 [sp. nov. in Plant, 2020]
**Diagnosis**. A yellowish species obscurely darkened on scutum medially and on scutellum and mediotergite. Best recognised by characters of the male terminalia, with cercus dilated apically and surstylus apically L-shaped. Description. Male: body length 2.4 mm; wing length 2.0 mm. Head. Slightly elongate. 1.4X long as deep, upper occiput making smooth curve where meeting vertex, hardly bulging. Black with paler dusting. Antenna and mouthparts whitish. One pair of minute reclinate ocellar setulae; ~ 3 pairs of smaller verticals. Antenna with postpedicel ~ 2.5–3.0X long as wide, stylus of similar length. Thorax. Yellow; scutum with vaguely darkened broad median stripe, at centre of which is much narrower and somewhat darker median stripe; scutellum and mediotergite brownish; small dark marks ventrally between insertion points of front coxae, obliquely about anterior spiracle, immediately in front of base of wing and in sutures of laterotergite. All setae yellowish, minute; notopleural very small; scutellum with pair of minute hairs on disc. Legs. Whitish yellow, distal tarsal segments hardly darker. C1 ~1.2X longer than distance between base of C1 and C2. C1, C2 and C3 all with minute setulae. F1 ~1.1X long as C1, ~ 5X long as wide, slightly constricted on ventral face 0.2 from base; with two parallel rows of ~16 minute black denticles ventrally, either side of which is linear series of ~5–6 inconspicuous fine yellow bristles, noticeably stronger around 0.2 from base. T1 ~0.75X long as F1, slightly curved, ventral face shallowly concave bearing two rows of ~ 20 minute, sharply pointed, blackish spinose setulae; ventroapical spur weakly developed; distinct ventroapical erect black spinose seta present. Wing. Membrane darkened by greyish microtrichia on distal 0.9, paler at base. Veins greyish, paler at extreme base. R2+3 linear, joining C ~ 0.7–0.8X distance between ends of R1 and R4. Length of C between ends of R2+3 and R4 ~1.2–1.4X long as R4 . R4 linear, hardly S-shaped, angle with R5 at extreme base ~ 75°. R5 ~2.2X long as R4. R4+5 fork distal to M1+2 fork by ~1.5X length of R4. R5 and M1 almost linear, slightly but distinctly convergent distally. Cell bm+dm ending just beyond end of R1. Halter yellowish white. Abdomen. Black, paler basally, especially on sternites 1–3. All setulae small and dark, becoming slightly longer on posterior margins of distal segments. Terminalia black. Cercus (Figs 8–9) elongate, extending slightly beyond tip of epandrium, conspicuously expanded dorsoapically (Fig. 8); with numerous distinct setae. Epandrium (Fig. 8) bluntly pointed apically, with some moderately strong bristles

apically. Surstylus present, extending distinctly beyond tip of epandrium, broadly L-shaped distally. Hypandrium pointed apically, with fine tomentum and few distinct bristles. Phallus not examined. Female: similar to male but scutum with broad median stripe more clearly defined. T1 with ventroapical setae rather longer. Abdomen with sternites 2–5 more distinctly yellowish; terminalia not elongate, not ovipositor-like.
**Type material**. **HOLOTYPE** ♂: **THAILAND**, Mae Hong Son Province, Ban Muang Phaeum, Phaeum River, 649 m, 19.5854°N, 98.30049°E, 4.iv.2019, netted A.R. Plant (QSBG). PARATYPES: 1♀, same data as holotype.
**Etymology**. The specific epithet honours Pairot Pramual in recognition of consistent support for my explorations of Thailand's Empididae fauna.
**Remarks**. The yellowish thorax with variably dark median stripe on the scutum recalls *H. systoechon* or *H. acutata*. However the thoracic markings of these species (and very probably of this species) are very variable and identification must rely on examination of the male terminalia. The male cercus is greatly dilated apically resembling that of *H. songsee* Plant, which has a very distinctive black and yellow pattern on the thorax, quite unlike the new species. A generally similar L-shaped apex of the surstylus occurs in other southeast Asian species (e.g., *H. attenuata, H. furcata, H. yunnanensis, H. digitata, H. oretenebraea* sp. nov. *and H. samoha* sp. nov.). *Hemerodromia pairoti* sp. nov. is known only from a calcareous river in the extreme northwest of Thailand.

### *Hemerodromia samoha* sp. nov. (Figs 10–11) [sp. nov. in Plant, 2020]
**Diagnosis**. A species with brownish or yellowish thorax with wing membrane and veins mostly dark. The cercus is elongate and crenulated dorsally with numerous long bristles and the upper lobe of the surstylus is distinctly L-shaped.
**Description**. Male: body length 2.2 mm; wing length 1.7 mm. Head. Rather elongate. 1.6–1.8X long as deep; upper occiput rather linear in lateral view making smooth curve where meeting vertex, not bulging. Black dusted greyish, antenna and mouthparts pale whitish yellow. One pair of minute reclinate ocellar setulae; 3–4 pairs of vertical setulae. Antenna with postpedicel ~2.5–3.0X long as wide, stylus of slightly shorter. Thorax. Yellowish with scutum posteriorly, scutellum and mediotergite brownish; dark markings ventrally between insertion points of front coxae, obliquely about anterior spiracle, immediately in front of base of wing and in sutures of laterotergite. All setae yellowish, minute, but notopleural distinct, scutellars apparently absent. Legs. Whitish yellow, distal tarsomeres of all legs hardly darker. C1 ~1.3–1.4X longer than distance between base of C1 and C2; C1, C2 and C3 with all setulae minute. F1 ~1.0–1.1X long as C1; 4–5X long as wide, slightly constricted on inner face at 0.25 from base. F1 ventrally with two rows of ~5–6 yellow spines, inconspicuous except 1–2 near base stronger; between which are two rows of ~23 (anteroventral series) and ~18 (posteroventral series) minute black denticles. T1 ~0.7X long as F1, slightly curved, ventral face shallowly concave bearing two rows of ~20 minute, sharply pointed, blackish spinose setulae; ventroapical spur weakly developed; distinct ventroapical erect black spinose seta present. Wing. Membrane darkened by blackish microtrichia on distal 0.9; proximal 0.1 contrastingly yellowish white. Veins blackish on distal 0.9 except about base of R1 and R4; pale on proximal 0.1. R2+3 linear, joining C ~ 0.7X distance between ends of R1 and R4. Length of C between ends of R2+3 and R4 ~1.7X long as R4. R4 hardly S-shaped, angle with R5 at extreme base ~ 75–80°. R5 long, ~3.0X long as R4. R4+5 fork distal to M1+2 fork by ~1.5X length of R4. R5 and M1 almost linear, distinctly convergent distally. Cell bm+dm ending just beyond end of R1. Halter yellowish white. Abdomen. Yellow but tergites 2–7 blackish. Setae mostly small and inconspicuous. Terminalia blackish. Cercus (Figs 10–11) elongate, extending beyond tip of epandrium, apically expanded and crenulate with numerous conspicuous bristles on crenulations apically. Epandrium (Fig.10) bluntly pointed apically, some distinct bristles distally. Two elongate processes emerging beyond tip of epandrium; upper process (upper lobe of surstylus?) large, L-shaped; lower process (lower lobe of surstylus?) with small black leaf-like appendage apically. Hypandrium strongly arched, lacking

distinct setae. Phallus not examined. Female: similar to male but abdomen with sternite 7 black, terminalia elongate, somewhat ovipositor-like.

**Type material**. **HOLOTYPE** ♂: **THAILAND**, Satun Province, Wang Sai Thong waterfall, 92 m, 7.09092°N, 99.909274°E, 13.v.2019, netted A.R. Plant (QSBG). PARATYPES:1♀, same data as holotype (QSBG); 2♂, 1♀, Nakhon Si Thammarat Province, Nan Sawan waterfall, 331 m, 7.89195°N, 99.78899°E, 11.v.2019, netted A.R. Plant (QSBG).

**Etymology**. The specific epithet honours Abdullah Samoh, fellow student of Empidoidea.

**Remarks**. The colour of the thorax of this species varies greatly from predominantly yellow with dark markings, to predominantly brownish and the species is probably best recognised by its distinctive male terminalia. The upper elongate process (here interpreted as the upper lobe of surstylus following Plant 2015) is distinctly L-shaped recalling particularly *H. furcata* Grootaert, Yang & Yang and *H. yunnanensis* (both widespread in Thailand) and *H. digitata* Grootaert, Yang & Saigusa (a species known from China, Yunnan) although many Asian species have apically inflated structures that despite being variously shaped, are probably analogous, and may be characteristic of a species-group within *Hemerodromia* (Plant 2015). Leaf-like appendages on the tip of the lower lobe of the surstylus are found in several regional species including *H. anisoserrata* Plant, *H. betalutea* Plant, *H. fusca* Yang &Yang, *H. ocellata* Plant and *H. yunnanensis*. The general pattern of colouration of the thorax and wing and crenulated dorsal process on the cercus of *H. samoha* sp. nov. recall *H. namtokhinpoon* Plant, a stenotopic tufa species only known from Loei province. The combination of cercus shape and form of the surstylus should enable identification of this species. *Hemerodromia samoha* sp. nov. is known from two tufa waterfall localities in the south of Thailand.

# 4.2 Environmental Preferences, Assemblage Structure and Distribution of *Hemerodromia*

### 4.2.1 *Locations*

Environmental data were analysed for 1,695 specimens of *Hemerodromia* that were identified to species-level.
Samples sites comprised

- tufa ecosystems (46)
- calcareous streams or rivers lacking tufa formations (23)
- springs (4)

Locations, names and elevation of sampling sites are indicated in Table 1 and mapped in Fig 12B. Two tufa sites were sampled on 5 separate occasions, 6 were visited twice and one non-tufa stream was visited twice. All other sites received a single visit. For analysis of assemblage composition, data for all sampling events at each site were pooled while environmental influences were investigated using all unique site-date sampling events.

### 4.2.2 *Environmental Variables at Sampling Sites*

Table 2 lists details of environmental variables observed / measured. The following summarises the observations.

- Elevation of sampling localities ranged from 26–1250m (mean=388±29, n=73) but lowland sites predominated (91% ≤ 700m).
- All sites were in forest, forest edge or forest remnants with deciduous – dipterocarp associations prominent in lowland sites but with strong influence of evergreen trees in immediate vicinity of watercourses and at higher elevations. Canopy cover varied from 10–100% (mean = 83 ± 2, n=73) and was ≥50% for 93% of sites.
- The relatively homogeneous structure of stream and rivers enabled reliable estimation of mean depth and mean flow ranging from 4–903 cm and 0.15–0.32 $m.s^{-1}$ respectively. However, tufa systems were extremely structurally heterogeneous, characterized by tufa accretion barriers, bosses and cones over which water flowed at high velocity (either as waterfalls or as fast films), but with largely still and sometimes deep pools upstream of these features. Steeply inclined but relatively uniform channels sometimes interconnect different hydrological features (especially on large well-developed tufa-cones) and there may be multiple flows forming a braided system. Mean depth (varying from 3–1540 cm) and mean flow (0.07–0.44 $m.s^{-1}$) measurements reported here do little to describe this complex hydrology. Similarly, analysis of the contribution of silt, sand, gravel, cobbles and rock (or tufa-encrusted rock) to the benthos was strongly influenced by heterogeneous hydrology.
- Water temperature at sample sites varied from 18.6–28.6°C and was weakly negatively correlated with elevation (26–1250m) in tufa systems (range 18.6–28.6, mean=24.4±0.3, $r^2$=0.415, n= 83) and between 62–1096m in non-tufa waters (range 18.6–28.5, mean=24.7±0.6, $r^2$=0.590, n= 25).

- Temperature was very weakly correlated ($r^2$=0.310, n= 87) with decreasing latitude (6.73–19.86°N) in tufa systems and in non-tufa waters ($r^2$=0.167, n= 25) at 7.59–19.58°N.

- Conductivity of tufa and non-tufa waters ranged from 200–793 µS.cm$^{-1}$ (mean= 399±18, n=57) and 39–765 (mean= 365±42, n= 27) respectively. In tufa and non-tufa systems there were extremely weak positive correlations of conductivity with elevation ($r^2$=0.182, n=57; $r^2$=0.286, n=27) temperature ($r^2$= 0.194, n=58; $r^2$= 0.208, n=25) and pH ($r^2$=0146, n=48; $r^2$=0.104, n=21) respectively. In both tufa and non-tufa systems, pH was unrelated to elevation ($r^2$= 0.028, n=48; $r^2$=0.068; $r^2$=0.068, n=18) or temperature ($r^2$=0.001, n=50; $r^2$=0.004, n=18).
- Seasonal variations in pH, conductivity and temperature were explored for all sites north of 14°N where the monsoon climate exerts strong seasonal variations in temperature and precipitation (in the south of Thailand, climatic seasonality is far less pronounced). Conductivity and pH decreased to minima in April–June coincident with the onset of the rainy season whereas temperature was probably maximum slightly later in the year (Fig 13). At the two sites which received 5 visits, there were variations in measured pH (8.3–8.5, 8.2–8.6), temperature (22.2–28.8, 22.6-25.7°C) and conductivity (285–409, 320-426 µS.cm$^{-1}$) that were approximately consistent with the overall seasonal variation.

### 4.2.3 *Environmental preferences of Hemerodromia*

- Ordination with MDS (Fig. 14) of an initial selection of primary habitat variables (pH, conductivity, temperature, presence of tufa; proximity to waterfalls and fast films) showed clear and separate grouping of tufa (*Tu1* & *Tu2* in Fig. 2) and stream / river localities lacking tufa (*Ri1* & *Ri2*), although neither was homogeneous and spring sites (*Spr*) clustered together.
- Bray-Curtis similarity between *Hemerodromia* assemblages on tufa and non-tufa sites was 0.246; 6 species were restricted to tufa, 3 were only found in the absence of tufa and 8 were found in both situations.
- K-dominance plots (Fig 15) indicated that species abundance distribution in both tufa and non-tufa assemblages were dominated by a small number of species (for example, *Hcon* and *Hfus* comprised 42% and 22% of total abundance at tufa sites while for non-tufa sites *Hfus* and *Hyun* comprised 51% and 22% respectively).

Table 3 further explores the occurrence of *Hemerodromia* spp. in relationship to the 6 primary habitat variables (pH, conductivity, temperature, presence of tufa; proximity to waterfalls and fast films). It is seen that. -

- *Hcon*, *Hana*, *Hnam*, *Hore*, *Hdep* and *Hsam* were confined to locations with strong tufa deposits (in Table 3, the proportion of individuals confined to tufa localities $P_t$ = 1,), all more or less restricted to the general vicinity of tufa waterfalls (high $P_w$), and more specifically, were only found in close proximity (≤ 1 m) to fast flowing films (high $P_{ff}$) of water flowing over tufa. There is of course likely to be some covariance between waterfall, fast film and tufa parameters but they retain usefulness as markers of specialist microhabitat within the tufa ecosystem.
- Although there were few significant differences (Table 3) between mean values of temperature, conductivity and pH tolerated by species, *Hfus* and *Hyun*, were found to occur in significantly broader ranges (P<0.001, Table 3) of these three environmental variables. Moreover, *Hfus* and *Hyun* were only weakly associated with tufa systems (low values of $P_t$, $P_{ff}$ and $P_w$) and these two species, along with *Hacu*, *Hani*, *Hflav* and *Hfur* are considered to be generalist eurytopic species (see Discussion).

- Several other species with low values of $P_t$, $P_{ff}$ and $P_w$ that occurred within relatively narrow ranges of pH, temperature and conductivity were only found on and downstream (*Hdem*, *Hzet*, *Hali*) or entirely downstream (*Hmen*, *Hpai*) of tufa systems and they are probably adapted more to calcareous waters than specific tufa habitats.

The influences of elevation, mean flow rate, mean depth, canopy coverage and physical characteristics of the benthos (sand, gravel, cobbles, silt, rock/tufa surface) at tufa sites were explored using CCA (Fig. 16) It was seen that. –

- The eigenvalues of the first two axes were 0.0201 and 0.0032 explaining 72.7% and 11.7 % respectively of the species relationship with these environmental variables and a Monte Carlo permutation test indicated that ordination was statistically significant (first axis $p<0.015$).
- The proportion of rock and sand in the benthos and canopy cover were important contributors to Axis 1. The presence of gravel in the benthos was the most important component of Axis 2 but there were influences of mean flow, depth and silt, although elevation was relatively unimportant.
- Most species occurred on the left side of the triplot indicating that canopy cover and a benthos comprised of rock or cobbles were favoured although *Hcon* ordinated on the right side, suggesting a preference for sand substrates and less importance of canopy cover.
- Most the species (excepting *Hano*) clearly identified as tufa specialists in Table 3 were grouped closely to Axis 1indicating that gravel, mean flow, depth, silt elevation were of minor importance to them, unlike generalist species such as *Hfus*, *Hyun* and *Hfur* that were more influenced by parameters of Axis 2.

### 4.2.4 *Assemblage structure and distribution*

Cluster analysis of assemblage composition (Fig 12A) and geographical distribution (Fig 12B) indicated weak clustering of communities from geographically related localities, but with week bootstrap support. Assemblages from Trat and Satun provinces in the far south of Thailand were retrieved in Cluster A (in Fig 12A). Data from more northerly provinces was mostly retrieved in Cluster B with some resolution of northern, western and lower northeastern assemblages in clusters B1, B2 and B3 respectively. Similarity between assemblages decayed with increasing distance between them (Fig 17).

### 4.2.5 *Phenology*

- Although sampling effort was uneven (fewer samples were taken mid wet season (June – September) it is clear (Table 4) that adults of various *Hemerodromia* species were active for at least 10 months of the year.
- Species richness (*S*) was greatest during April – May and relative abundance (*A**) was greatest in March – June. Because water temperature varied seasonally in the north (Fig. 1) and might trigger emergence (Ivković *et a*l., 2013), correlations between abundance and temperature were investigated for the more data-rich *Hemerodromia* species sampled north of 14°N. Abundance ($\log_{10}$ transformed) was uncorrelated with temperature for *Hano* ($r^2=0.066$, n=15), *Hcon* ($r^2=0.054$, n=25) and *Hfus* ($r^2=0.026$, n=51); very weakly positively correlated for *Hnam* ($r^2=00.242$, n=11) and *Hzet* ($r^2=0.313$, n=7); very weakly negatively correlated for *Hyun* ($r^2=0.337$, n=22).

#### 4.2.6 *Anthropic influences*

Tourism facilities were present at 45 (98%) of the 46 tufa systems studied (and also at many other tufa sites which were not selected for sampling as they were considered unsuitable or inaccessible due to tourism). Visitor facilities varied from simple access paths and viewing areas, but others were transformed by widespread construction of networks of paths, bridges, concrete-dammed bathing pools and other visitor facilities. Artificial flow diversion and use of concrete or sandbags to create / enlarge tufa barriers and other tourist-friendly features were noted. Selective removal of large trees (resulting in improved viewing and access) occurred at some sites and at more popular locations, the shrub and herb layers were completely destroyed by trampling (at one site, the vegetation was actively removed by site managers). Human trampling of tufa resulting in polishing, compaction and disintegration occurred where large numbers of bathers used tufa systems but notices forbidding climbing on tufa formation or use of detergents when bathing were sometimes present. Poaching by cattle was observed at two sites where the tufa surface had become deeply fractured and pitted and faecal contamination was present in pools.

Anthropic changes to hydrology were observed at 44 (96%) of the 46 tufa systems and all springs examined. Water was abstracted for agriculture or drinking (piped directly from resurgences or from pools constructed upstream of the tufa formations) and was sometimes diverted from source to maintain a useful depth in bathing pools, with consequent drying of natural channels and destruction of the aquatic environment. A complete lack of surface water observed at two tufa cones was attributed by local knowledge to over-abstraction during ongoing drought in 2020 (the sites have apparently never ‘run dry’ in living memory).

Many tufa sites or their feed-streams were located in agricultural settings (e.g. cultivation of rice, betal and oil palm). Sediment-laden agricultural runoff deposited deep layers of silt in the pools behind tufa barriers at most sites although at some locations, siltation was tentatively attributed to industrial runoff from cement manufacture.

## 4.3 Population genetic structure of *Hemerodromia conspecta*

### 4.3.1 *Mitochondrial genealogy*

- A median-joining (MJ) network calculated from 263 COI sequences of *H. conspecta* indicated a high level of genetic structure (Fig 18) with major breaks between lineages comprised of haplotypes rather tightly clustered within the well sampled geographically defined populations A–D.
- The two poorly sampled geographical regions, Chiang Mai and Mae Hong Son, represented another well separated lineage (designated E in Fig 18).

### 4.3.2 *Variation of DNA sequences*

- A total of 43 haplotypes were distinguished in 259 COI sequences obtained from four sample-rich populations of *H. conspecta* in Loei (A, n = 114), Lampang + Chiang Rai (B, n = 56), Phrae (C, n = 25) and Tak + Kanchanaburi (D, n = 64) provinces (Table 5).
- A further four haplotypes were resolved from under sampled populations in Chiang Mai (E, n = 3) and Mae Hong Son (F, n = 1).
- Overall haplotype diversity across all populations was 0.782 (Table 5) but for individual populations varied from a very low value of 0.0692 in population A to 0.8333 in C.
- Genetic variation measured as nucleotide diversity was also lowest in A (0.00013 ± 0.00027) but an order of magnitude higher in populations B and C with an overall value of 0.0227 ± 0.0115 (Table 5).

### 4.3.3 *Demographic history*

- Mismatch distribution analysis of the four major lineages, A, B, C and D (E and F were omitted because of small sample sizes) of *H. conspecta* revealed unimodal mismatch graphs (Fig. 19) that were not significantly different from the simulated data under the sudden population expansion model (Rogers & Harpending 1992).
- Low nucleotide diversity associated with high haplotype diversity as exhibited in lineages B, C and D (Table 5) is also consistent with the sudden population expansion model. Although population expansion parameters of lineage A (negative values of Tajima'D and Fu' Fs and no significant difference of sum-of-square-deviation (SSD) and Harpending's raggedness index from the simulated data under the sudden expansion model) might also indicate population expansion, the very low haplotype diversity of this lineage is a strong indication that it has experienced a profound population bottleneck.
- The timing of population expansion estimated from mismatch distribution was strongly dependent on generation time *T*. Using values of *T* ranging from 1 – 12 generations/year we estimated divergence times as 99000 – 9900 years ago (lineage A), 116000 – 11600 (B), 98500 – 9800 (C) and 105000 – 10500 (D).

# 5 SUMMARY DISCUSSION and CONCLUSION

**1. The genus *Hemerodromia* predominates.** Species of other genera of aquatic Empididae (*Clinocera, Chelipoda* and *Achelipoda*) were rare and their data was not analysed in detail.

**2. Five species of *Hemerodromia* were described as new**.

**3. One species known** previously from China (Yunnan) was **found for the first time**, in northern Thailand.

**4. Eleven species of *Hemerodromia*** had previously been found in Thailand

**5. Local and regional-scale endemism was apparent at species-level in *Hemerodromia***
Excepting eurytopic species of widespread occurrence in Asia (*H. fusca, H. yunnanensis*), most species had narrow distribution ranges, for example:-

- *H. namtokhinpoon* Some species are entirely restricted to to small geographical areas, e.g. *H. namtokhinpoon* (Loei).
- Some species are exclusively southern, e.g. *H. deprimatura* (Phang Nga) or *H. samoha* (Satun &Nakhon Si Thammarat.
- Some species are exclusively northern, e.g. *H. aliaextricata*, *H. pairoti* (Mae Hong Son)
- Some species have widespread by extremely patchy distributions in the north and west, e.g. *H. conspecta* in Loei, Lampang, Chiang Rai, Chiang Mai, Mae Hong Son, Tak and Kanchanaburi.
- Some species demonstrate extreme range disjunctions, e.g. *H. oreotenebraea* which occurs in the northern provinces of Loei, Lampang, Phrae, Chiang Rai but with an isolated population 1,000 km to the south in Phang Nga.

**6. There are no are cold water refugia in which persists a relict fauna from historical cold climatic phases.**
The hypothesis that cold-adapted *Clinocera* or other aquatic Empididae may have been historically marooned in cold groundwater resurgences at low elevation remains unsupported. Limestone streams in Thailand's karsts are probably mostly allogenic with short subsurface residence times so water temperature predominately equilibrates with atmospheric temperatures at different elevations. In this study, the temperature of emergent waters decreased from 28.6 – 18.6°C along an elevation gradient of 26 – 1,250 m. Cold water resurgences were not found at low elevation and it is highly unlikely that cold-adapted organisms are present in resurgent waters in Thailand.

**7. Some species are very strictly associated with tufa biotopes.**
Six species only occurred in very close association with active tufa biotopes. These species are strongly niche conservative and stenotopic for tufa, occurring in waters with relatively high pH and conductivity. –

- *H. conspecta*
- *H. anomala*
- *H. namtokhinpoon*
- *H. oretenebraea*
- *H. deprimatura*
- *H. samoha*

**8. Some species require calcareous waters but are not confined to tufa.**
Five species were only found on and downstream or entirely downstream of tufa biotopes where they tolerated specific ranges of relatively high pH and conductivity. They were not found in non-calcareous, acidic or neutral habitats and they are probably adapted more to calcareous waters than specific tufa habitats. –

- *H. menghaiensis*
- *H. zetalutea*
- *H. aliaextriata*
- *H. demissa*
- *H. pairoti*

All other taxa recorded had no apparent association with tufa or calcareous water. They were generalists that are able to survive in the conditions present at such sites but are tolerant of wider ranges of pH and conductivity and other environmental parameters

**9. Tufa specialists species require shallow fast flowing water**
Adults of all six strict tufa specialists (*H. conspecta, H. anomala, H. namtokhinpoon, H. oretenebraea, H. deprimatura and H. samoha*) were very strictly confined to dense shade within 1 m of shallow films of water flowing over actively accreting tufa. Adults have very poor dispersal ability and it is likely that they do not move more than a few meters from their larval habitats. We conclude that immature stages are likely found in 'fast films' of water on tufa.

**10. Canopy cover, bankside vegetation and benthos are critical to tufa specialists**
Ordination employing a range of environmental variables (elevation, mean flow rate, mean depth, canopy coverage and physical characteristics of the benthos; sand, gravel, cobbles, silt, rock/tufa surface) indicated that although 'fast films' were important to tufa specialist species, they also favoured closed canopy conditions and extensive bankside vegetation (although *Hemerodromia conspecta* also tolerated more open conditions). Solid benthic substrates (tufa-accreted rock surfaces) were critical to most tufa specialists.

**11.** Species abundance distribution in both tufa and non-tufa assemblages indicated **dominance by a small number of species**. (not necessarily calcareous specialists

**12.** Tufa habitats are revealed as being particularly interesting as they support highly specialized range-restricted species but their communities are **threatened by tourism and agricultural activities**

**13.** A **high level of genetic structure of populations of *H. conspecta*** was indicated by median-joining (MJ) haplotype network calculated from COI sequence data. **5 Distinct lineages** were present in geographically close localities in Loei, Lampang + Chiang Rai, Phrae , Tak + Kanchanaburi and Chiang Mai + Mae Hong Son.

**14.** Haplotype diversity (*h*), nucleotide diversity ($\pi$) and pairwise comparisons of $F_{ST}$ a suggested considerable **differentiation among populations** but evolutionary distance judged from the Kimura 2-parameter was too low (0.98–4.58) to establish any lineage as a discrete species, but was considered as **evidence of ongoing processes of allopatric speciation**.

**15.** Mismatch distribution analysis and low nucleotide diversity associated with high haplotype diversity in three lineages (Lampang + Chiang Rai, Phrae, Tak + Kanchanaburi) was consistent with the **sudden expansion model** but very low haplotype diversity of a fourth lineage (Loei) provided strong indication that it had **historically experienced a profound population bottleneck.**

**16.** Long path lengths in the MJ network suggested **prolonged isolation of the four major lineages** (Lampang + Chiang Rai, Phrae, Tak + Kanchanaburi and Loei) and mismatch distribution analysis indicated that their populations experienced a **late Pleistocene expansion (~10,000 – 100,000 bp) that was complete by the early Holocene**, entirely consistent with evidence for a climatically driven period of late Cenozoic tufa deposition followed by late Quaternary decline.

**17.** The allopatric distribution of geographically fragmented and genetically distinct lineages supports the view that **vicariant diversification is driving active processes of speciation and microendemism occurring within multiple microrefugia set within a wider matrix of unsuitable habitats**.

# 6. FULL DISCUSSION

## 6.1 *New species*

Five new species of *Hemerodromia* were described and one other (previously known from China) was found for the first time in Thailand raising the total species richness of the genus in Thailand from 25 to 31. This increase supports the suggestion by Plant (2015) that the number of known species will be increased considerably if specific habitats are examined in detail (rather than general sampling). Only two specimens of a (probably) undescribed *Clinocera* were found. A revision of this genus is planned for the near future (with B. Sinclair, Ottawa, Canada) but it is very unlikely that future work will reveal high species richness of thei genus in the lowlands (see later)

## 6.2 *Taxa and assemblage composition*

Of the two aquatic subfamilies of Empididae, Hemerodromiinae predominated with the genus *Hemerodromia* accounting for 99.5% of all specimens. Clinocerinae were represented by just two examples of *Clinocera* collected from a warm water site at 22.5°C. The hypothesis that cold-adapted *Clinocera* or other aquatic Empididae may have been historically marooned in cold groundwater resurgences at low elevation remains unsupported. Limestone streams in Thailand's karsts are probably mostly allogenic with short subsurface residence times so water temperature predominately equilibrates with atmospheric temperatures at different elevations. In this study, the temperature of emergent waters decreased from 28.6–18.6°C along an elevation gradient of 26–1250 m and Martin Ellis (pers. comm.) found a similar gradient of air temperature within caves at different altitudes (29.2–14.0°C at 5–1750 m). Cold water resurgences were not found at low elevation and it is highly unlikely that cold-adapted organisms are present in resurgent waters in Thailand.

*Hemerodromia* was diverse (17 spp. of which 5 were new species) and abundant (1850 individuals). Composition of species assemblages at tufa and non-tufa sites were markedly different as judged by a Bray-Curtis similarity and they were separately resolved with MDS. Six species (*Hcon*, *Hano*, *Hnam*, *Hore*, *Hdep* and *Hsam*) were obligately associated with tufa systems; two species (*Hmen*, *Hpai*) were associated exclusively with non-tufa calcareous streams and rivers and three species (*Hdem*, *Hzet*, *Hal*i) occurred both on tufa and non-tufa calcareous sites. The remaining 6 species (*Hacu*, *Hani*, *Hflav*, *Hfur*, *Hfus* and *Hyun*) are considered to be generalists and have previously known associations with non-calcareous habitats (Plant, 2015), Two generalist species (*Hfus* and *Hyun*) were widespread (occurring in 47[64%] and 21[29%] of sample sites) and they contributed to high dominance (species with high abundance) indicated by the shape of the species abundance distribution at both tufa and non-tufa sites although *Hcon* was most abundant at tufa sites. Five species were very rare with ≤5 specimens sampled and 8 species (*Hali*, *Hani*, *Hdep*, *Hfla*, *Hmen*, *Hnam*, *Hpai* and *Hsam*) were found at ≤2 sites suggesting range restriction.

*Hnam* was entirely restricted to the two geographically close (within 2km of each other) tufa localities on Carboniferous-Permian limestones of the Saraburi Group in Loei province (Lower Northeast) from where is was described (Plant, 2015) and is clearly a narrow-range endemic species. *Hali*, *Hpai* and *Hdem* were restricted to the far north in

Chiang Mai and Mae Hong Son. *Hmen* was also found only in Mae Hong Son but it was originally described from a limestone site 360 km NE in Yunnan China (Yang & Yang, 2004). *Hani* and *Hfla* are rare species, not restricted to calcareous sites and probably with northern distributions (Plant, 2015) while two prominent species on tufa, *Hcon* and *Hano* are widespread north of 14°N but show no selective affinity for limestone of any particular age or formation. *Hdep* and *Hsam* are tufa species that are apparently endemic to the South. *Hore* had a disjunct distribution with populations in the North and Lower Northeast and distantly in the South. Geographical distributions of aquatic *Hemerodromia* have likely been shaped by historical fragmentation and connectivity of hydrological networks, mountain orogenesis, development of a monsoon climate and glacial intercessions (Plant, 2015; Plant *et al*., 2012). The distributions of calciphile species reported here are consonant with biogeographic groupings and origins advanced by Plant (2015) for *Hemerodromia* in Thailand ("northern endemic"," southern endemic" "northern lowland endemic", "widespread lowland" etc.).

### 6.3 *Environmental preferences*

A striking characteristic of the obligately tufa-associated *Hemerodromia* is that the adults were all captured close to waterfalls, and more particularly, almost exclusively within 1 m of fast films of water flowing over tufa. It is hypothesised that immature stages are associated with these fast-flowing waters. As with most aquatic insects, association of immature Empididae with their aquatic micro-habitat more properly requires their determination *in situ* (usually impossible) or use of emergence data (Smith et al, 2003; Gerecke et al., 2011). Emergence data could not be collected in this study and the hypothesis rests solely on exclusive association of adults with specific habitat. Adult *Hemerodromia* are weak fliers with poor dispersal ability (Wagner, 1997) seldom, if ever found away from bankside vegetation. While in temperate habitats, adult *Hemerodromia* may occasionally disperse to vegetation as much as 10 m from watercourses, fidelity to bankside vegetation is very strong in the tropical fauna. Adults are extremely sensitive to desiccation and as indicated earlier, they have probably survived historic increases in aridity by vertical migration into cool, damp habitat (Plant *et al*., 2012) whereas lowland species persist by remaining precisely confined to deep shade in very close proximity to water and are intolerant of more open and less humid conditions (Plant, 2015). It appears that in Thailand, strictly tufa-associated species probably have immature stages restricted to shallow films of fast flowing water on tufa cones, barriers and waterfalls, close to where adults are found.

Ordination with CCA supported the conclusion that rock (or more precisely a tufa-accreted rock surface) was important for many species of *Hemerodromia* at tufa sites. The physical characteristics of the benthos (rocks and coarse particulates rather than fine particulates) may have important roles in regulating the composition of assemblages of certain aquatic insects Dumnicka *et al*., 2007) including aquatic Empididae (Ivković *et a*l., 2012, 2015) and faster water flow is also preferred by many aquatic Empididae (Harper, 1980; Wagner & Gathmann, 1996; Ivković *et a*l., 2012 ) In this study, CCA suggested a low importance of water velocity, perhaps because the measurement used was an average of different flows at different parts of a tufa system, rather than at specific micro-environments (fast films). This is however, the first report of a unique association of Empididae communities with fast films on tufa, but the finding is entirely consistent with known associations with high velocity flows and solid benthic substrates discussed above.

CCA indicated that many species of *Hemerodromia* (with the exception of *Hcon*) favoured closed canopy conditions. Canopy vegetation greatly influences light availability and temperature thereby impacting on primary production (Danks & Williams, 1991; Death & Collier, 2010) with consequent effects on the larvae of benthic invertebrates at different trophic levels (Davies et al., 2005, Murphy et al., 1981; Progar & Moldenke, 2009; Ivković *et a*l., 2012). Canopy and bankside vegetation presumably also help maintain humid conditions, which, as discussed above, are critical to adult *Hemerodromia*. It is notable that although *Hcon* showed a preference for sites with a more open canopy, it was never found away from deeply shaded habitats

**6.4 *Seasonality***

In northern temperate climates, *Hemerodromia* are largely univoltine, the single adult emergence peak of each species occurring from between April and September (Wagner & Gathman, 1996; Plant, 2003; Ivković *et a*l., 2012). In tropical Fiji (Plant & Sinclair, 2008) and in the equatorial Amazon Basin (Câmara et al. 2014) members of the genus can be found throughout the year, although individual species may have seasonal adult activity maxima. In Thailand, even though some species can be found throughout the year, others are more or less restricted to the cold season or to the wet season (Plant, 2015) and this is perhaps reflected in this study where maximum species richness occurred in March – April (just before commencement of the wet season) and during the early cold, dry season during October – November (Table 4). Species with limited emergence span contribute to these seasonal maxima (e.g. 10 species were only found in 1–2 months of the year) and most likely have a single generation each year. However, other species, most noticeably *Hfus* and *Hcon* (which were reported during 10 and 8 months respectively) are active for a longer span of time and their reproduction is likely multivoltine or iteroparous. Profound seasonal changes in composition and structure of communities has also been noted for other aquatic Diptera in Thailand (Srisuka et a., 2015; Pramual & Wongpakam, 2010).

Seasonal or diurnal changes of temperature and photoperiod regulate emergence in many temperate insects (Corbet, 1964; Hynes, 1976; Tauber & Tauber, 1975) and increase in temperature appears to be the major trigger of daily emergence patterns of at least some species of *Hemerodromia* (Ivković *et a*l., 2013). Intra-annual shifts in precipitation and temperature profoundly influence the biota of tropical biotopes and their effects are particularly strong in the monsoonal tropics which experience approximately alternating wet / dry and hot / cool seasons. This study found an annual temperature range of 10°C (18.6–28.6°C) which is similar to the range (10.1°C, 6.8–16.9°C) reported (Ivković *et a*l., 2007) for summer temperatures at a Mediterranean karst spring where water temperature is an important determinant of assemblage structure. Although abundance of *Hnam*, *Hzet* and *Hyun* was correlated with water temperature, albeit very poorly, and *Hcon*, *Hfus*, *Hyun*, *Hnam*, *Hacu* and *Hore* may have favoured discrete temperature ranges (Table 1) the role of temperature requires further study and evaluation. Variations in discharge patterns in different years have a strong influence on community composition of aquatic Empididae (Wagner & Gathmann, 1996; Wagner & Schmidt, 2004) and it is expected that aquatic Empididae will also respond to seasonal climatic cycles. This was not investigated here but it was noticed that no aquatic Empididae were found on tufa systems that had dried out completely.

### 6.5 *Endemism*

The similarity of *Hemerodromia* assemblage composition decayed with distance between tufa sites. Although CA resolved clusters that were associated geographically with populations from the Lower Northeast, North, West and South, they were weakly resolved and with poor statistical support. There was no obvious relationships between assemblage clusters and underlying geology as, for example, multiple geographical clusters could be superimposed on the widespread presence of Carboniferous-Permian rocks at many sites of the North, Lower Northeast and South. Therefore, despite the existence of regionally endemic species noted earlier, there was little evidence of assemblages bounded by discrete geography or surface geology but assemblages that were further apart were less similar than those that were closer together.

### 6.6 *Anthropic influences*

Tourism and water abstraction were almost ubiquitous at tufa sites with obvious and sometimes massive effects including (1) physical destruction and polishing of tufa (2) thinning of the forest canopy and eradication of ground-level vegetation (3) physical modification of watercourses resulting in altered flows (e.g. creation of artificial bathing pools) and (4) unnaturally extreme discharge events (e.g. diverting water at source, bypassing and thereby desiccating its natural channel). Waterfall tourism, often in protected areas, is an important recreational activity and business in Thailand. For example, Chuanchom & Popichit (2016) estimated that the 712763 visitors to the Erawan Waterfall National Park in 2015 generated a revenue of 90.6 million baht and that visitor activities in the waterfall areas were the major management challenge. Although National environmental quality standards, acknowledge certain threats to waterfalls (Ministry of Natural Resources & Environment, 2015) there appears to be little recognition of the specific biotic and geological importance of active tufa systems.

Abstraction of water for irrigation and siltation appear to be important agricultural influences on tufa systems. Water is abstracted upstream resulting in the tufa drying out. Sediment-laden agricultural runoff is deposited as deep layers of fine silts in pools upstream of tufa barriers. The source of this sediment appears to be topsoil erosion and may be remote from the site of deposition (e.g. water laden with eroded topsoil may sink at one locality and resurge several km distant at a tufa site). Although the larvae of some aquatic Diptera prefer silts (Drake, 1985) they are not favoured by aquatic Empididae (Ivković *et a*l., 2012; Wagner & Gathmann, 1996) and agricultural runoff is likely to have negative influences on their assemblages.

The true extent of anthropic influences on tufa sites is certainly greater than indicated here because, as mentioned earlier, many potential sampling-sites were rejected on account of being incorporated into Buddhist temple features or by springs being capped and water piped directly from the resurgence.

### 6.7 *Population Genetics of Hemerodromia conspecta*

*Hemerodromia conspecta* was selected as a model for investigating genetic structure as is a tufa stenotope with discrete population demonstrating range disjunctions of a considerable geographic area.

A high level of genetic diversity among populations of *H. conspecta* in northern and western Thailand was revealed by molecular genetic data based on mtDNA COI sequences. The species is stenotopic for tufa environments and has an obligate association with shallow water flowing rapidly over tufa surfaces (Plant 2020). Although it occurs across ~ 110,000 $km^2$ of northern Thailand north of the line from 14.6°N,98.9°E and 17.0°N,101.7°E, the mean distance (± SE) between the 12 known locations (sampled in this study) was 291 ± 20 km (n = 66), reflecting the considerable geographical separation between individual patches of rare habitat. The species is consequently entirely restricted to a highly fragmented and dispersed habitat. Pairwise comparisons of $F_{ST}$ between populations of *H. conspecta* were entirely consistent with the hypothesis that genetic differentiation between them is being driven by stenotopy (specialised niche requirement), presumably because gene flow is inhibited by extensive matrix areas of unsuitable habitat separating suitable tufa habitat patches. This result is consistent with the Mantel test which effectively excluded genetic structure of populations arising as a simple consequence of geographical distance. Rather, differentiation of allopatric populations has been influenced by vicariant events linked to habitat specialisation.

The MJ network also reinforces the hypothesis of limited geneflow between populations. Four lineages (Figs 1 & 2) occurred within discrete geographical areas; Lineage A from Loei, B from Lampang and Chiang Rai, C from Phrae and D from Tak and Kanchanaburi. Lineages from Mae Hong Son and from Chiang Mai (lineage E) were also resolved in the MJ network but with a low number of samples. Kimura 2-parameter (Table 4) is a useful proxy for evolutionary distance and which varied from 0.98 – 4.58; values too low compared with those reported as being useful to establish specific identity in insects (Previšić *et al*. 2009; Pramual & Adler 2014; Morinière *et al*. 2019). Lineages of *H. conspecta* are thus unlikely to represent cryptic species although distances between them likely reflect ongoing processes of speciation.

Long path lengths in the MJ network are suggestive or long isolation of the different lineages. We employed mismatch distribution analysis to obtain approximate population expansion times of different lineages, even though the approach is subject to many potential sources of error (Schenekar & Weiss 2011). One major source of error is generation time (*T*). We do not know *T* for any species of *Hemerodromia* and we can only tentatively infer from limited knowledge of biology that a complete cycle of egg to adult might take a minimum of one month. Many northern temperate species of the genus are univoltine with adults of different species having a seasonal duration of activity spanning ~ 0.1 – 0.5 years (Plant 2003) while in tropical Thailand, adult activity is also often highly seasonally correlated with different phases of the monsoon and spans 0.1 – 0.7 years, although a few species occur throughout the year (Plant 2015). We have observed adult *H. conspecta* (including freshly emerged teneral adults) over ~8 months and conclude that it has multiple generations each year. It must also be noted that *T* has probably varied historically in response to climatic cycles as temperature is a powerful determinant of adult phenology of aquatic Empididae (Ivković *et al*. 2013). In our estimation of the timing of population expansion, we calculated lower and higher limits using *T* = 1 generation/year and *T* = 12 generations / year, thereby dating divergence of lineage A at 99000 – 9900 bp, B at 116000 – 11600 bp, C at 98500 – 9800 bp and D at 105000 – 10500 bp. These dates cover a vast range of time, but we can be reasonably confident that expansion took place sometime during the last 100,000 years of the Pleistocene and was concluded by the early Holocene (commenced 11700 bp).

As mentioned earlier, low nucleotide diversity and high haplotype diversity of lineages B, C and D (and the star-shaped pattern evident in Fig. 2), are consistent with a sudden expansion model in which populations are actively differentiating. Lineage A is strikingly different with very low haplotype diversity suggestive of it having experienced a population bottleneck. Drastic reduction of populations in response to environmental crises is an expected feature of very highly specialised and transient habitats, especially in organisms with low dispersal abilities and strong niche conservatism. Reduced populations surviving a bottleneck may experience genetic drift (Miller and Lambert 2004; Nyström *et al*. 2006), subjecting them to non-adaptive radiation (Gittenberger 1991; Hardy *et al*. 2016;) and, because they are inherently less genetically varied than their progenitors, can experience founder effects that influence the 'direction' of speciation. Given presumed low vagility of *H. conspecta* and its extreme stenotopy in rare and highly dispersed habitat patches that are surely ephemeral on evolutionary timescales, population bottlenecks are likely to be important drivers of the species' evolution.

The allopatric distribution of genetically distinct lineages in geographically remote locations indicates that vicariance has played a role in diversification. The lineages are not sufficiently differentiated to warrant recognition as discrete species, but our findings do reveal likely ongoing processes of speciation and microendemism occurring within multiple microrefugia set within a wider matrix of profoundly unsuitable habitats. Most fossil tufa deposits date from the Late Cenozoic (Ford & Pedley 1996). Given that tufa is an accretion product of carbonates reworked during karstification, it is perhaps unsurprising that both karstification and tufa formation favour similar climatic conditions during warm pluvial climatic phases (Liu, 1997; Liu *et al*. 2001; Andrews 2006; Capezzouli *et al*. 2014;). The Late Cenozoic "golden age" of tufa formation would have been promoted by climatic cycles linked to Pleistocene glacial events, and in Asia, also by intensification of a seasonally wet monsoon climate since the Miocene (Liu *et al*., 2011). Population expansion time estimates for *H. conspecta* lineages at approximately 10000 to 100000 bp are entirely congruous with a Late Cenozoic "golden age" of tufa, although it should be recognised that even then, tufa must have been uncommon and of local occurrence in the wider matrix. Isolation of *H. conspecta* populations in fragmented palaeorefugia might have commenced during unfavourable Pleistocene climatic events. By the mid-late Quaternary, tufa systems were declining and becoming inactive or eroding in many places (Goudie *et al*.1993; Glover & Robertson 2003; Fubelli *et al*. 2013) and fragmentation of populations might have been largely complete by the start of the Holocene (11700 bp) during which Southeast Asia probably experienced intense and prolonged droughts (Griffiths *et al*. 2020) that would not have been conducive for tufa formation..

Further work should investigate the population history and genetic structure of other stenotopic tufa species. This would facilitate better correlations between patterns of allopatric diversification and the spatial and temporal characteristics of microendemic tufa species with their Pleistocene and Holocene history.

### 6.8 *Concluding remarks*

This work is the first demonstration of rich assemblages of aquatic Empididae from tropical karst waters in Asia. Tufa habitats are revealed as being particularly interesting as they support highly specialized range-restricted species and their populations appear to have experienced long isolation since around the start of the Holocene. Their genetically distinct communities are are fragmented and vulnerable to extinction, especially as they are threatened by tourism and agricultural activities. Data on influences of environmental parameters on the occurrence of *Hemerodromia* was gathered from sampling of adults on few occasions at many sites. Although this approach yielded much information, future studies should concentrate on sampling immature forms (or emerging adults) from a limited number of sites throughout at least one year in order to closely associate environmental variables with *exact* sites of larval development.

## 7. ACKNOWLEDGEMENTS

This work was generously funded by the **Thailand Research Fund (DBG6180024).** The not inconsiderable task of finding suitable locations for sampling was eased somewhat by information supplied by Chaiporn Siripornbul (Department of Mineral Resources, Bangkok, Thailand) and especially Martin Ellis (Shepton Mallet Caving Club, UK) who very kindly supplied a copy of the Thailand cave database in Speliobase 2.1.3 and also made available data on air temperature in Thai caves. Clive Burrett (University of Mahasarakham, Thailand) provided helpful discussion of geology. My wife Busayanan is thanked for her hard work as a driver, informal field technician, and problem-solver.

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## 9. CAPTIONS FOR FIGURES

Figs 1 – 6
Terminalia of; 1 – 2 *Hemerodromia aliaextricata,* 3 – 4 *Hemerodromia deprimatura,* 5 – 7 *Hemerodromia oreotenebraea*

Figs 8 – 11
Terminalia of; 8 – 9 *Hemerodromia pairoti*, 10 – 11 *Hemerodromia samoha*

Fig 12A & 12B
Cluster analysis of *Hemerodromia* assemblages at tufa sites using unweighted pair-group average and Jaccard similarity. Bootstrapping was performed with 1000 resamples and the percentage of replicates where each cluster is still supported is shown at the nodes. Number and colour of termini correspond with individual site codes and regional groupings respectively, as indicated in Fig 12B
Provinces are.- *C*, Chayaphum; *CM*, Chiang Mai; *CR*, Chiang Rai; *K*, Kanchanaburi; *L*, Loei; *La*, Lampang; *Lo*, Lopburi; *MHS*, Mae Hong Son; *N*, Nan; *NR*, Nakhon Ratchasima; *NST*, Nakhon Si Thammarat; *P*, Phitsanulok; *Ph*, Phrae; *Pn*, Phangnga; *Pt*, Phatthalung; *S*, Saraburi; *Sa*, Satun; *ST*, Surat Thani; *T*, Tak; *Tr*, Trang.

Fig 13
Monthly 3-point circular mean ±SE of pH, temperature and conductivity at all sites at latitudes ≥14°N. Curves were fitted by eye.

Fig 14
Ordination with MDS, using Bray-Curtis similarity, of key habitats (pH, conductivity, temperature, presence of tufa; proximity to waterfalls and fast films) of *Hemerodromia* assemblages in Thailand. *Tu1*, *Tu2* tufa localities; *Ri1*, *Ri2*, localities lacking tufa; *Spr*, spring sites.

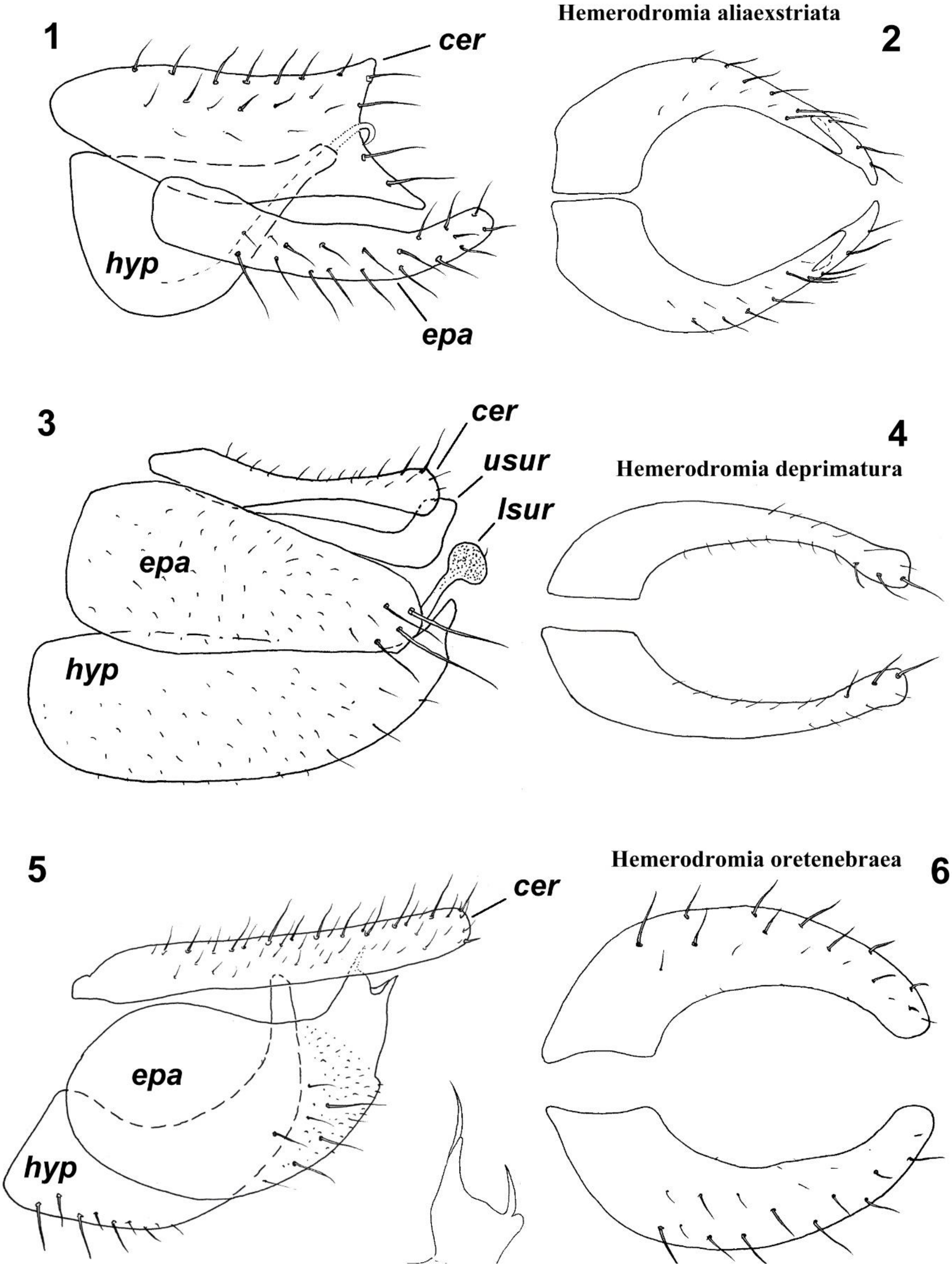
Fig 15
K-dominance plot of species abundance distribution of *Hemerodromia* species at sites with and without tufa. The relative cumulative abundance is plotted against $\log_2$ species rank (from highest to lowest).

Fig 16
First two axes of CCA ordination of 13 *Hemerodromia* species in tufa habitat sites using 9 environmental variables.- elevation, mean depth, mean flow rate, canopy cover and physical characteristics of the benthic substrate (sand, gravel, cobbles, silt, rock/tufa surface).

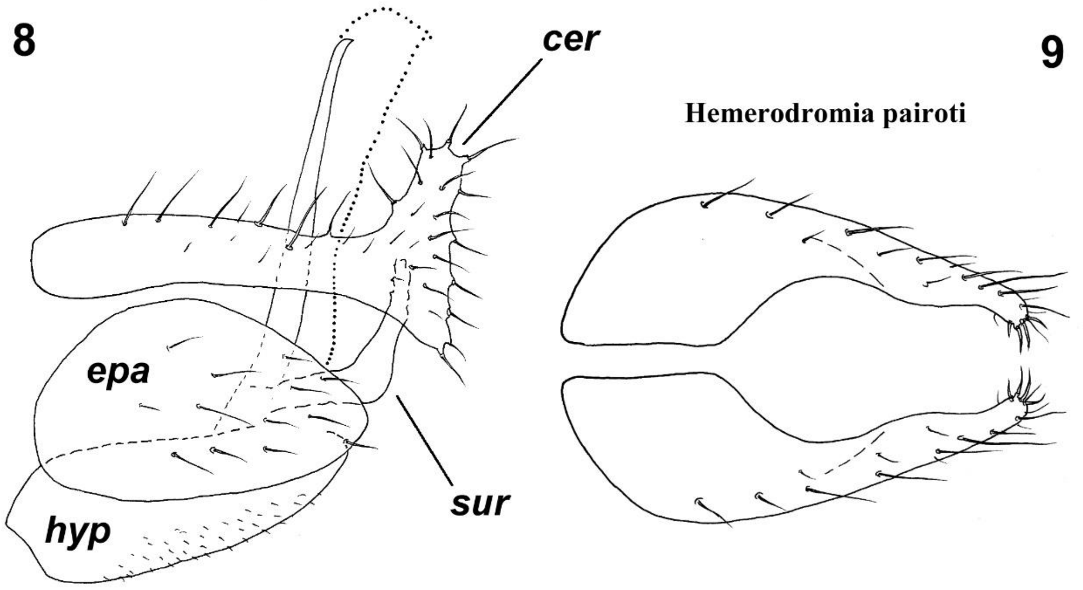
Fig 17
Plot of mean distance ±SE between tufa sites against Jaccard similarity of their *Hemerodromia* assemblages.

Fig. 18. Mitochondrial DNA genealogy indicated by a median joining network constructed from 263 COI sequences of *Hemerodromia conspecta.* Each haplotype is represented by a circle. Sizes of circles are relative to number of individuals sharing specific haplotypes and

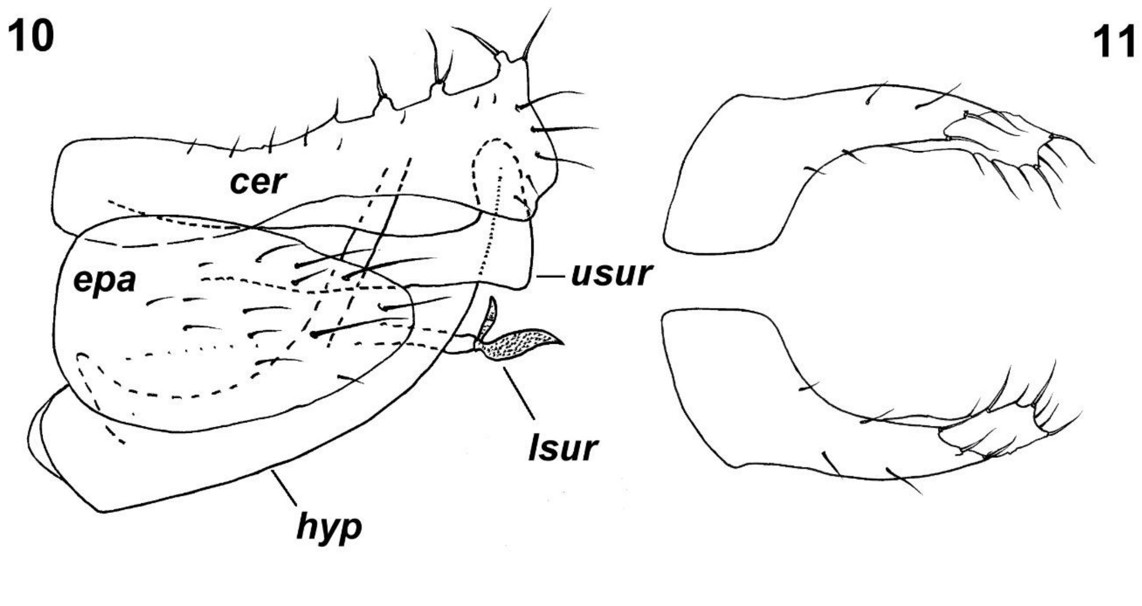
the colour of circles denotes different geographical provinces. A–E indicates different lineages.

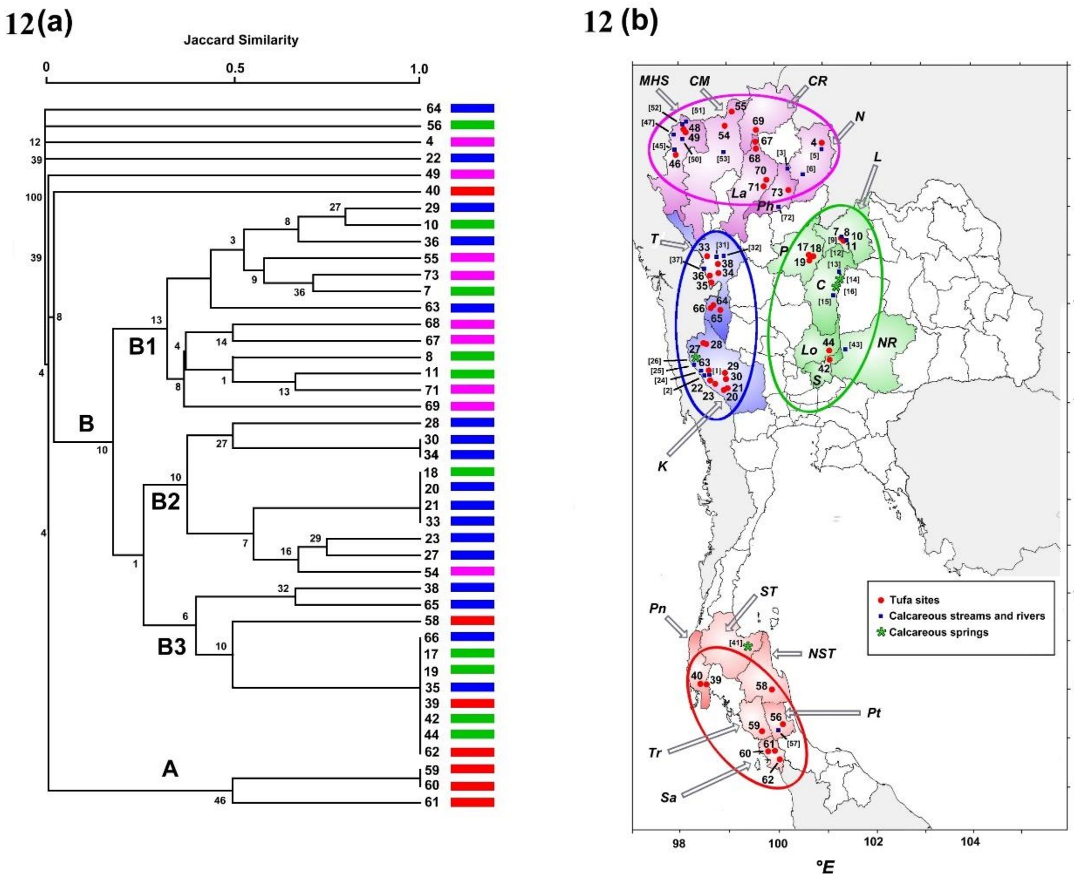


Hemerodromia pairoti




Hemerodromia samoha




12(a)
Jaccard Similarity
0
0.5
1.0
B1
B2
B3
B
A
12 (b)
MHS
CM
CR
N
L
T
P
C
NR
Lo
S
K
La
Ph
Pn
ST
NST
Pt
Tr
Sa
Tufa sites
Calcareous streams and rivers
Calcareous springs
°N
°E

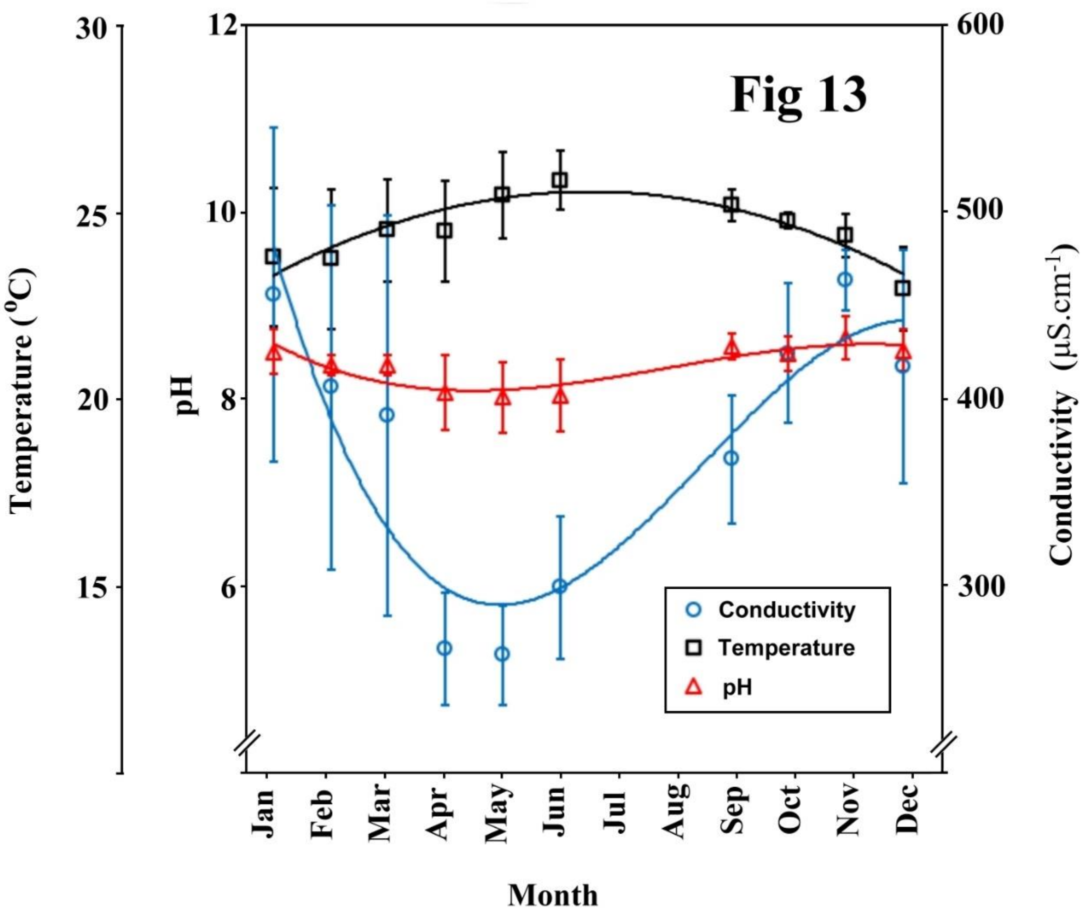


Fig 13
Temperature (°C)
pH
Conductivity (µS.cm-1)
Month
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Conductivity
Temperature
pH
30
25
20
15
12
10
8
6
600
500
400
300

Fig 14

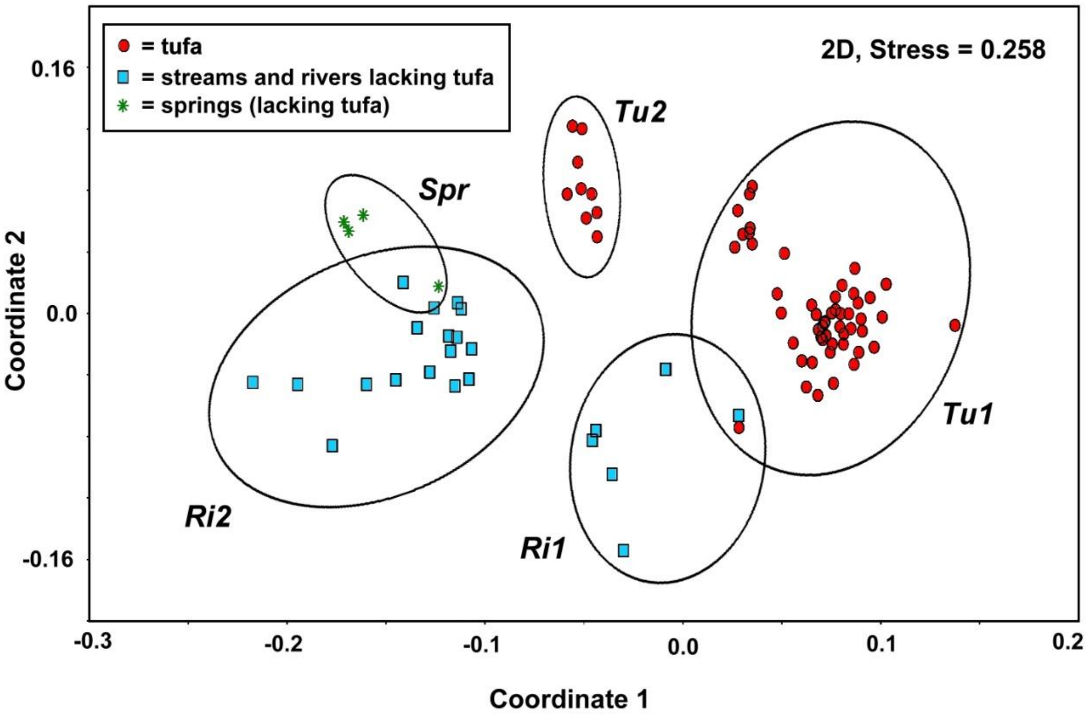
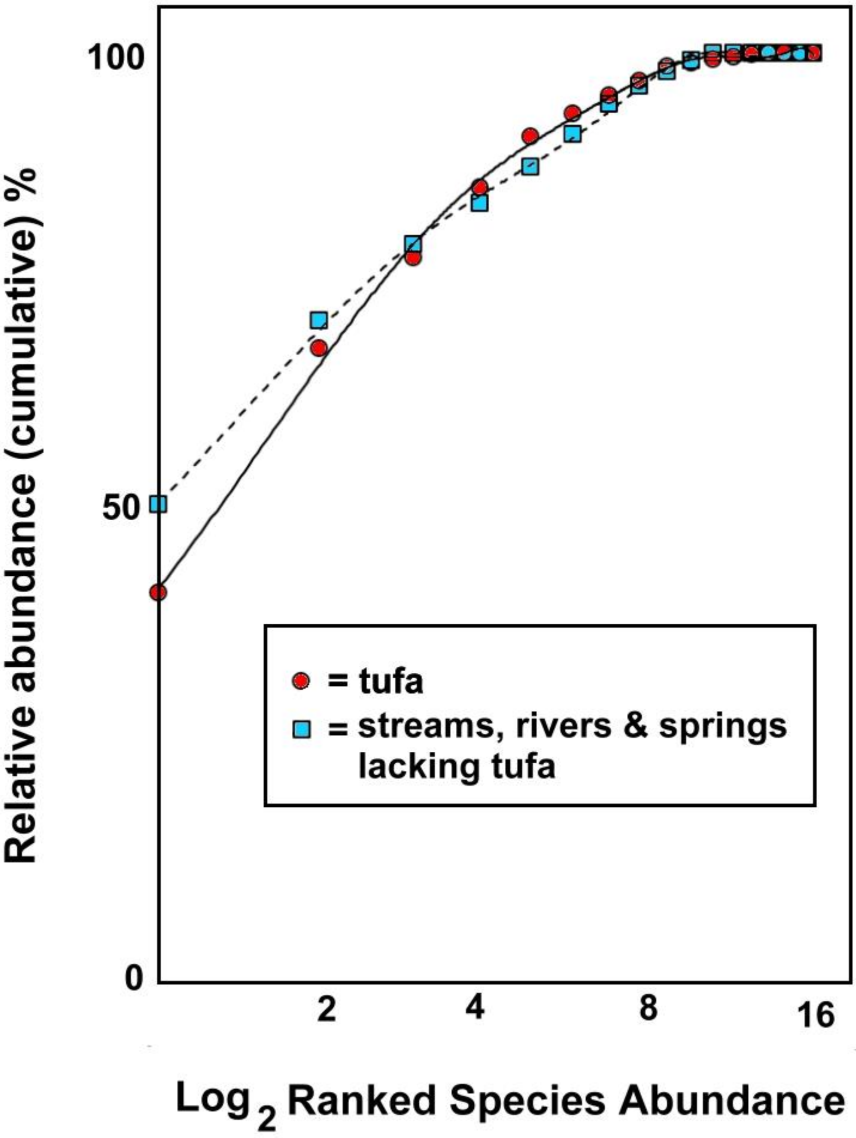

= tufa
= streams and rivers lacking tufa
= springs (lacking tufa)
2D, Stress = 0.258
Tu2
Spr
Tu1
Ri2
Ri1
0.16
0.0
-0.16
Coordinate 2
-0.3
-0.2
-0.1
0.0
0.1
0.2
Coordinate 1

Fig. 15


100
50
0
2
4
8
16
Relative abundance (cumulative) %
Log 2 Ranked Species Abundance
= tufa
= streams, rivers & springs lacking tufa

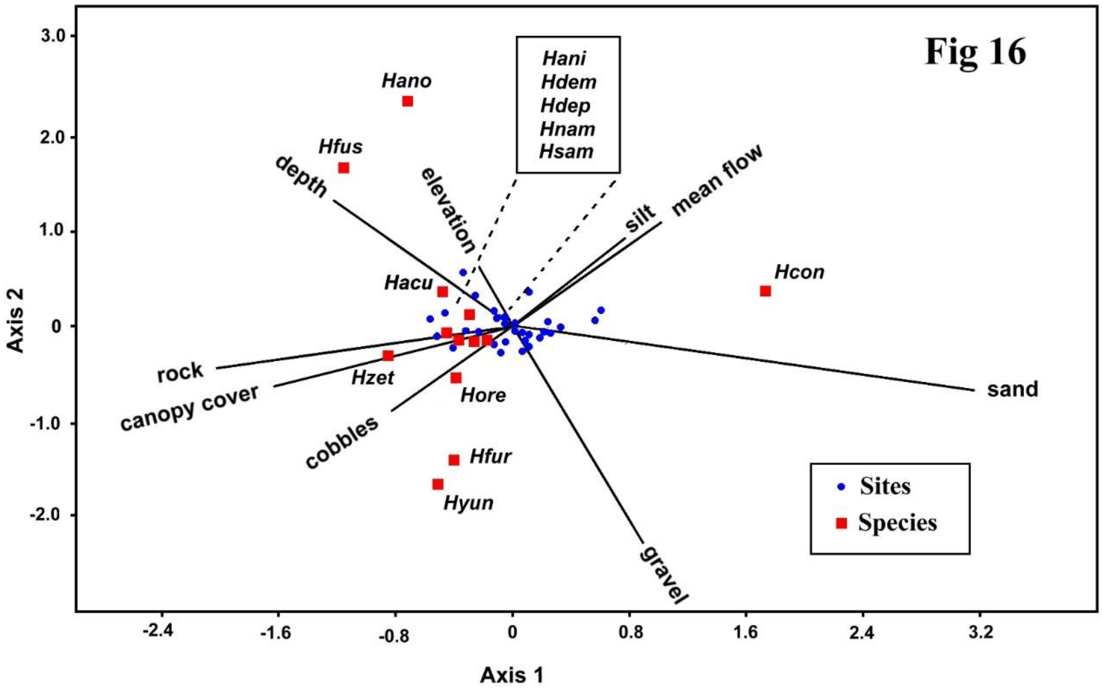


Fig 16
Hano
Hfus
Hani
Hdem
Hdep
Hnam
Hsam
depth
elevation
silt
mean flow
Hacu
Hcon
rock
canopy cover
Hzet
Hore
cobbles
sand
Hfur
Hyun
gravel
Sites
Species
Axis 2
3.0
2.0
1.0
0
-1.0
-2.0
-2.4
-1.6
-0.8
0
0.8
1.6
2.4
3.2
Axis 1

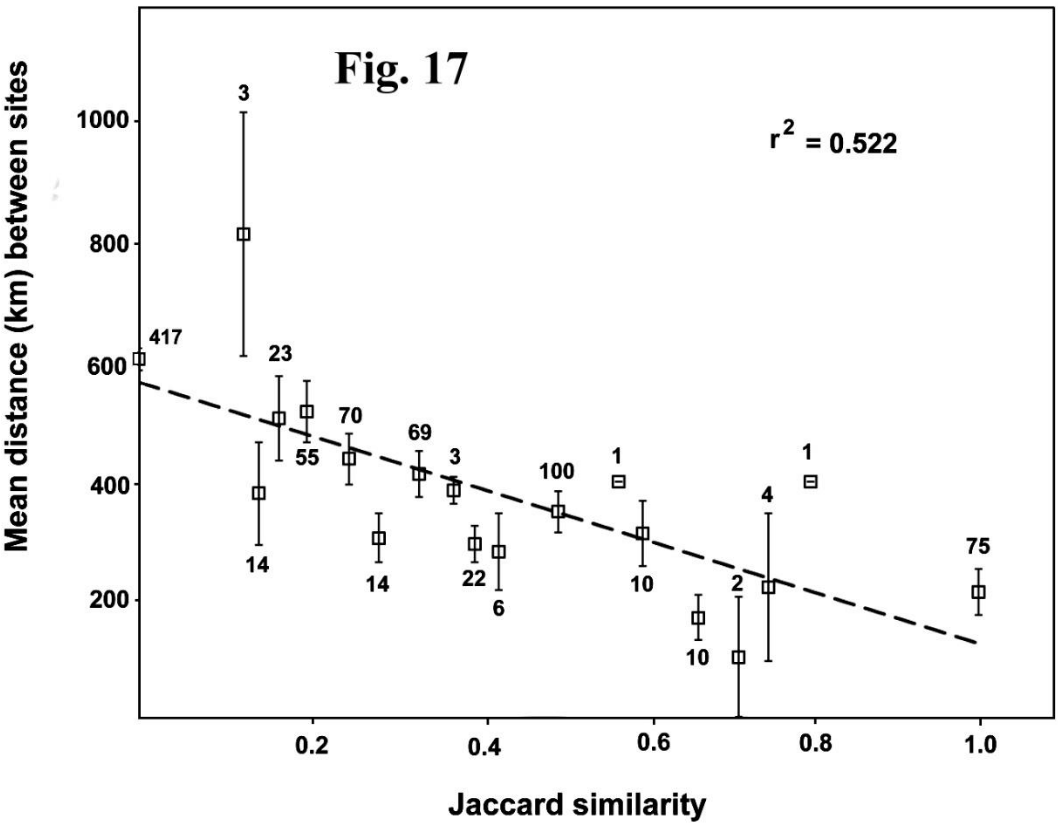
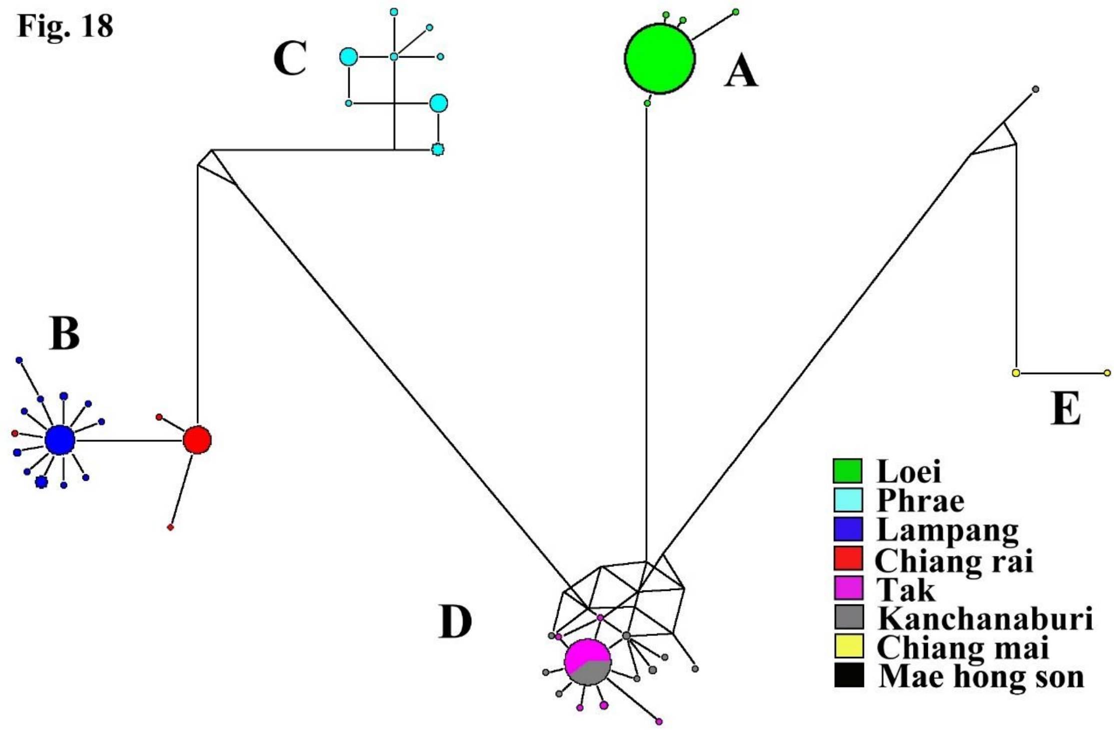
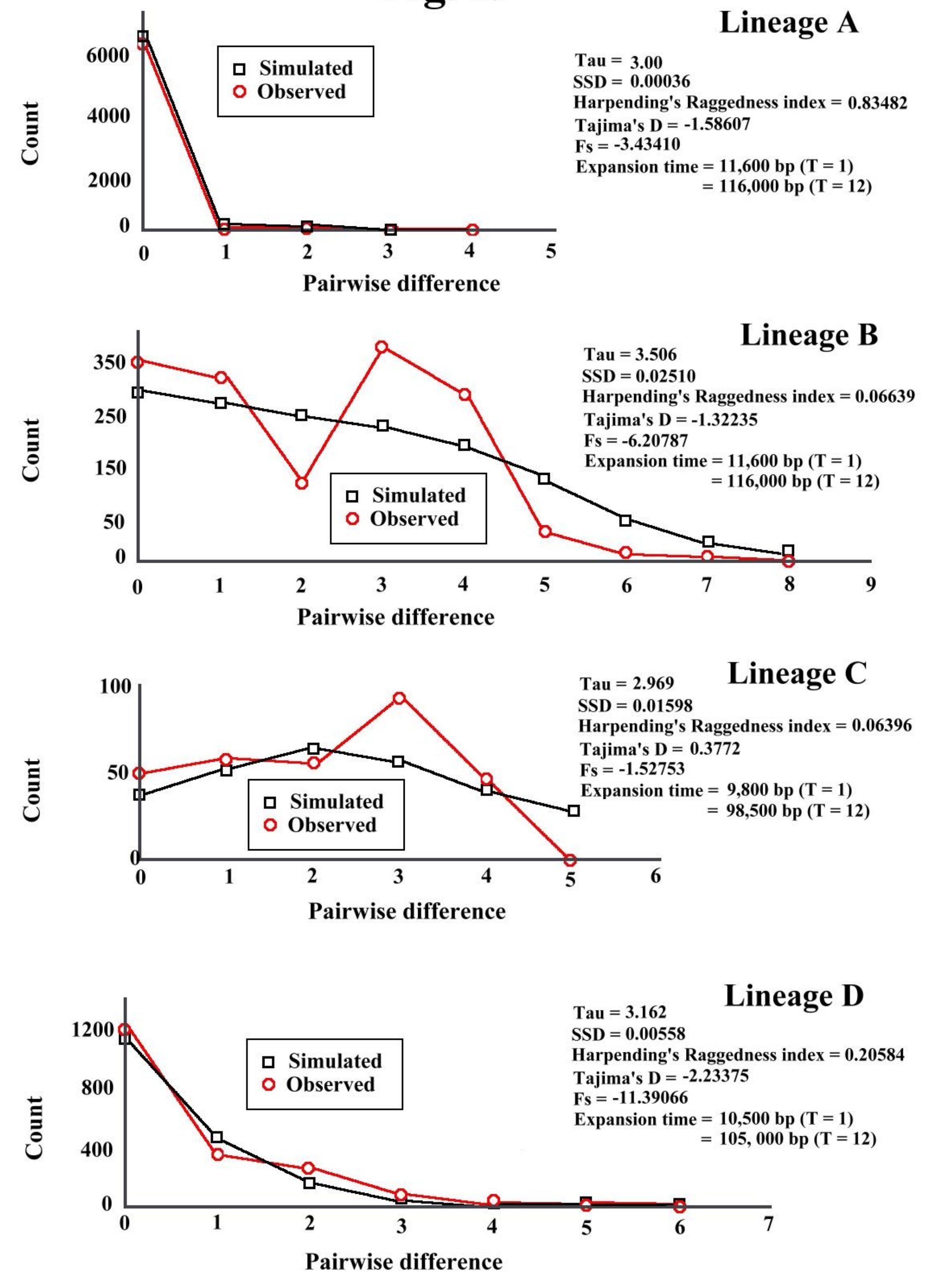


Fig. 17
$r^2$ = 0.522
Mean distance (km) between sites
1000
800
600
400
200
0.2
0.4
0.6
0.8
1.0
Jaccard similarity
417
3
23
55
14
70
14
69
3
22
6
100
1
10
10
2
4
1
75

Fig. 18


C
A
B
E
D
Loei
Phrae
Lampang
Chiang rai
Tak
Kanchanaburi
Chiang mai
Mae hong son

# Fig. 19


Lineage A
Tau = 3.00
SSD = 0.00036
Harpending's Raggedness index = 0.83482
Tajima's D = -1.58607
Fs = -3.43410
Expansion time = 11,600 bp (T = 1)
= 116,000 bp (T = 12)
Simulated
Observed
Count
6000
4000
2000
0
0
1
2
3
4
5
Pairwise difference
Lineage B
Tau = 3.506
SSD = 0.02510
Harpending's Raggedness index = 0.06639
Tajima's D = -1.32235
Fs = -6.20787
Expansion time = 11,600 bp (T = 1)
= 116,000 bp (T = 12)
Simulated
Observed
Count
350
250
150
50
0
0
1
2
3
4
5
6
7
8
9
Pairwise difference
Lineage C
Tau = 2.969
SSD = 0.01598
Harpending's Raggedness index = 0.06396
Tajima's D = 0.3772
Fs = -1.52753
Expansion time = 9,800 bp (T = 1)
= 98,500 bp (T = 12)
Simulated
Observed
Count
100
50
0
0
1
2
3
4
5
6
Pairwise difference
Lineage D
Tau = 3.162
SSD = 0.00558
Harpending's Raggedness index = 0.20584
Tajima's D = -2.23375
Fs = -11.39066
Expansion time = 10,500 bp (T = 1)
= 105, 000 bp (T = 12)
Simulated
Observed
Count
1200
800
400
0
0
1
2
3
4
5
6
7
Pairwise difference

## Table 1.

Sampling locations and number of taxa collected. Sample codes amended as a,b,c,d & e indicate a single site was sampled multiple dates indicated. Site types are.- Tu, streams & rivers with tufa present; St, streams and rivers without tufa formations; Sp, springs.

Provinces are.- *C*, Chayaphum; *CM*, Chiang Mai; *CR*, Chiang Rai; *K*, Kanchanaburi; *L*, Loei; *La*, Lampang; *Lo*, Lopburi; *MHS*, Mae Hong Son; *N*, Nan; *NR*, Nakhon Ratchasima; *NST*, Nakhon Si Thammarat; *P*, Phitsanulok; *Ph*, Phrae; *Pn*, Phangnga; *Pt*, Phatthalung; *S*, Saraburi; *Sa*, Satun; *ST*, Surat Thani; *T*, Tak; *Tr*, Trang.

| | | | | | | | Species abundance | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Site CODE | Type | Name | Province | Coordinates | Elevation (m) | Date | *Hcon* | *Hfus* | *Hano* | *Hfur* | *Hyun* | *Hnam* | *Hdem* | *Hzet* | *Hacu* | *Hore* | *Hani* | *Hmen* | *Hali* | *Hdep* | *Hsam* | *Hflav* | *Hpai* | *Clinocera sp.* | *Akan* | *Akha* | *Cnak* |
| 1 | St | Hin Dat | *K* | 14.6253° N, 98.7262° E | 79 | 11.ix.2018 | | 8 | | | | | | | | | | | | | | | | | | | |
| 2 | St | Di Chong Tong | *K* | 14.9983° N, 98.6203° E | 197 | 14.ix.2018 | | | | | 2 | | | | | | | | | | | | | | 1 | | |
| 2a | – | – | – | – | – | 26.xi.2018 | | | | | | | | | | | | | | | | | | | | | |
| 3 | St | unknown | *Ph* | 18.4635° N, 100.4911 °E | 394 | 04.x.2018 | | 1 | | | | | | | | | | | | | | | | | | | |
| 4 | Tu | Tun Ton | *N* | 19.2109° N, 101.0679 °E | 924 | 05.x.2018 | | 1 | | | | | | | | | | | | | | | | | 1 | | |
| 5 | St | Huai Nam Ton | *N* | 19.1976° N, 101.0766 °E | 1096 | 05.x.2018 | | | | 5 | | | | | | | | | | | | 3 | | | | | |
| 6 | St | unknown | *N* | 18.2795° N, 100.5043 °E | 1250 | 06.x.2018 | | | | | | | | | | | | | | | | | | | | 1 | |
| 7 | Tu | Pang Din (mid) | *L* | 17.0663° N, 101.7481 °E | 614 | 17.x.2018 | 44 | | 6 | | 1 | 8 | | 10 | | 4 | | | | | | | | | | | |
| 7a | – | – | – | – | – | 05.iii.2019 | 46 | 2 | 7 | | | 7 | | | | | | | | | | | | | | | |
| 7b | – | – | – | – | – | 26.vi.2019 | 25 | | | | | 3 | | | | | | | | | | | | | | | |
| 7c | – | – | – | – | – | 27.ix.2019 | 36 | | 1 | | | 5 | | | | | | | | | | | | | | | |
| 7d | – | – | – | – | – | 04.iii.2020 | 2 | 5 | 9 | | | 1 | | | | | | | | | | | | | | | |
| 8 | Tu | Pang Din (lower) | *L* | 17.0666° N, 101.7491 °E | 557 | 17.x.2018 | 24 | | | | | 15 | | | | | | | | | | | | | | | |
| 9 | St | Pang Din (higher) | *L* | 17.0646° N, 101.7483 °E | 613 | 15.x.2018 | | 9 | | | 4 | | | | | | | | | | | | | | | | |
| 10 | Tu | Suan Hom (upper) | *L* | 17.0464° N, 101.7616 °E | 592 | 16.x.2018 | 39 | | 3 | | | 2 | | | | | | | | | | | | | | | |
| 10a | – | – | – | – | – | 06.iii.2019 | 19 | 10 | 54 | | 1 | 23 | | | | | | | | | | | | | | | |
| 10b | – | – | – | – | – | 26.vi.2019 | 12 | 18 | 2 | | | 9 | | | | | | | | | | | | | | | |
| 10c | – | – | – | – | – | 27.ix.2019 | | | | | | | | | | | | | | | | | | | | | |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 10d | – | – | – | – | – | 04.iii.2020 | | | 3 | | | 1 | | | |
| 11 | Tu | Suan Hom (lower) | *L* | 17.0470° N, 101.7617°E | 576 | 17.x.2018 | 13 | 9 | | | | 3 | | | |
| 12 | St | Suan Hom (exit St) | *L* | 17.0530° N, 101.7656°E | 547 | 17.x.2018 | | 1 | | | | | | | |
| 13 | St | Nam Phut Tap Lao | *C* | 16.5446° N, 101.8531°E | 359 | 18.x.2018 | | | | | | | | | |
| 14 | Sp | Nam Phut Hin Lat | *C* | 16.6159° N, 101.8948°E | 238 | 19.x.2018 | | | | | 1 | | | | |
| 15 | St | Nam Phut Na Lao | *C* | 16.5954° N, 101.8980°E | 246 | 19.x.2018 | | 3 | | | | | | | |
| 16 | Sp | Ban Nam Un | *C* | 16.5780° N, 101.8692°E | 260 | 20.x.2018 | | | | | | | | | |
| 17 | Tu | Noen Maprang | *P* | 16.5972° N, 100.677° E | 121 | 15.xi.2018 | | 7 | | | | | | | |
| 18 | Tu | Noen Maprang | *P* | 16.5834° N, 100.6862°E | 66 | 15.xi.2018 | | 1 | | | 1 | | | | |
| 18a | – | – | – | – | – | 22.xi.2019 | | 5 | | | | | | | |
| 19 | Tu | Noen Maprang | *P* | 16.5841° N, 100.6858°E | 77 | 15.xi.2018 | | 40 | | | | | | | |
| 20 | Tu | SaiYok Noi | *K* | 14.2391° N, 99.0583° E | 134 | 23.xi.2018 | | 2 | | | 1 | | | | |
| 21 | Tu | Sai Yok Noi (source) | *K* | 14.2402° N, 99.0596° E | 136 | 23.xi.2018 | | 1 | | | | | | | |
| 22 | Tu | Sai Yok Lek | *K* | 14.4337° N, 98.8532° E | 65 | 23.xi.2018 | | | | | | | | | |
| 23 | Tu | Sai Yok No 2 | *K* | 14.4473° N, 98.8500° E | 41 | 24.xi.2018 | | 1 | | | | | | | |
| 23a | – | – | – | – | – | 30.x.2019 | | 5 | 1 | | 4 | | | | |
| 24 | St | Wat Pa Sunantha Wanaram | *K* | 14.5386° N, 98.8317° E | 155 | 24.xi.2018 | | | | | 1 | | | | |
| 25 | St | Thong Pha Phum (1) | *K* | 14.7475° N, 98.5797° E | 185 | 25.xi.2018 | | 16 | | | | | | | |
| 26 | Sp | Thong Pha Phum (2) | *K* | 14.7017° N, 98.5924° E | 198 | 25.xi.2018 | | 2 | | | | | | | |
| 27 | Tu | Krateng Jeng (1) | *K* | 15.0295° N, 98.6034° E | 173 | 26.xi.2018 | | 1 | | | 5 | | | | 1 |
| 27a | – | – | – | – | – | 30.x.2019 | | 6 | 2 | | | | | | |
| 28 | Tu | Krateng Jeng (2) | *K* | 15.0285° N, 98.6026° E | 178 | 26.xi.2018 | | | | | 2 | | | | 2 |
| 29 | Tu | Huai Mae Khamin (lower ) | *K* | 14.6385° N, 98.9911° E | 199 | 27.xi.2018 | 3 | 4 | 7 | | 1 | | | | |
| 29a | – | – | – | – | – | 01.xi.2019 | 7 | 20 | | | 2 | | | | |
| 30 | Tu | Huai Mae Khamin (upper) | *K* | 14.6413° N, 98.9800° E | 266 | 27.xi.2018 | 2 | 9 | | | 1 | | | | |
| 30a | – | – | – | – | – | 01.xi.2019 | | | | | 1 | | | | |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 31 | St | unknown | *T* | 16.7936° N, 98.9204° E | 720 | 12.xii.2018 | | | | | | | | | | | | | | | | | | | | | 3 |
| 32 | St | Lansang | *T* | 16.777° N, 99.0035° E | 323 | 13.xii.2018 | | | | | 23 | | | | | | | | | | | | | | | | |
| 33 | Tu | Mae KaSa | *T* | 16.8617° N, 98.6309° E | 225 | 15.xii.2018 | | 4 | | | 3 | | | | | | | | | | | | | | | | |
| 34 | Tu | Pa Wai | *T* | 16.5746° N, 98.8337° E | 736 | 16.xii.2018 | | | | | 2 | | | | | | | | | | | | | | | | |
| 35 | Tu | Nang Khruan | *T* | 16.4091° N, 98.6893° E | 358 | 16.xii.2018 | | 12 | | | | | | | | | | | | | | | | | | | |
| 36 | Tu | Tararak (lower) | *T* | 16.5699° N, 98.6948° E | 567 | 17.xii.2018 | 84 | 7 | | | | | | | | | | | | | | | | | | | |
| 36a | – | – | – | – | – | 28.xii.2019 | 94 | 9 | 14 | | | | | | | | | | | | | | | | | | |
| 37 | St | Tararak (upper) | *T* | 16.5703° N, 98.6952° E | 627 | 17.xii.2018 | | 6 | | | | | | | | | | | | | | | | | | | |
| 38 | Tu | "Muser" | *T* | 16.7257° N, 98.9146° E | 568 | 18.xii.2018 | | 6 | 1 | | | | | 2 | | | | | | | | | | | | | |
| 39 | Tu | Tao Tong | *Pn* | 8.4859° N, 98.5851° E | 26 | 13.i.2019 | | 13 | | | | | | | | | | | | | | | | | | | |
| 40 | Tu | Sa Nang Namora | *Pn* | 8.5106° N, 98.5414° E | 59 | 16.i.2019 | | | | | | | | | | 1 | | | | 5 | | | | | | | |
| 41 | Sp | Tham Khong Wat | *ST* | 8.9466° N, 99.5332° E | 97 | 17.i.2019 | | 1 | | | | | | | | | | | | | | | | | | | |
| 42 | Tu | Chet Sao Noi | *S* | 14.7294° N, 101.192° E | 165 | 20.ii.2019 | | 13 | | | | | | | | | | | | | | | | | | | |
| 43 | St | Huai Sai | *NR* | 14.7542° N, 101.2107 °E | 160 | 20.ii.2019 | | 4 | | | | | | | | | | | | | | | | | | | |
| 44 | Tu | Suan Madua | *Lo* | 14.9199° N, 101.2164 °E | 121 | 21.ii.2019 | | 2 | | | | | | | | | | | | | | | | | | | |
| 45 | St | Mae Nuam Luang | *MHS* | 18.8353° N, 98.0466° E | 849 | 01.iv.2019 | | 5 | | | 11 | | | | 16 | | | | | | | | | | | | |
| 46 | Tu | unknown | *MHS* | 18.8327° N, 98.0474° E | 855 | 01.iv.2019 | | | | | | | | | | | | | | | | | | | | | |
| 47 | St | Pang Tong | *MHS* | 19.5037° N, 97.9498° E | 953 | 02.iv.2019 | | 10 | | 4 | 8 | | | | 5 | | | | | | | | | | | | |
| 48 | Tu | Susa (1) | *MHS* | 19.4745° N, 98.1247° E | 312 | 03.iv.2019 | | 1 | | | | | 1 | | | | | | | | | | | | | | |
| 49 | Tu | Susa (2) | *MHS* | 19.4745° N, 98.1242° E | 305 | 03.iv.2019 | 1 | 6 | | | | | | | | | 4 | | 3 | | | | | | | | |
| 50 | St | Susa (river) | *MHS* | 19.4779° N, 98.1208° E | 306 | 03.iv.2019 | | | | | | | 3 | | | | 5 | | 4 | | | | | | | | |
| 51 | St | Phaeum River | *MHS* | 19.5854° N, 98.3004° E | 649 | 04.iv.2019 | | 16 | | | | | 3 | 6 | | | | | | | | | 2 | | | | |
| 52 | St | Mae lang | *MHS* | 19.5364° N, 98.2714° E | 632 | 04.iv.2019 | | 2 | | | | | 4 | | | | | 8 | | | | | | | | | |
| 53 | St | Mork Fa , Nam Sai | *CM* | 19.1137° N, 98.7717° E | 547 | 05.iv.2019 | | 36 | | | | | 1 | 4 | | | | | | | | | | | | | |
| 54 | Tu | Sri Sangwan | *CM* | 19.6272° N, 98.9533° E | 568 | 06.iv.2019 | | 11 | 15 | | 3 | | 21 | | | | | | | | | | | | | | |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 55 | Tu | Pong nan Dang | *CM* | 19.8638° N, 99.1116° E | 653 | 07.iv.2019 | 3 | 22 | 7 | | | | 5 | 7 | | | | | | | | | | |
| 56 | Tu | Keuan Nam Phut | *Pt* | 7.5899° N, 99.9128° E | 50 | 10.v.2019 | | | | | | | | | | | | | | | | | | |
| 57 | St | Tham Sumano | *Pt* | 7.5861° N, 99.8681° E | 62 | 10.v.2000 | | | | | | | | | | | | | | | | | | |
| 58 | Tu | Nan Sawan | *NST* | 7.8919° N, 99.7889° E | 139 | 11.v.2019 | | 15 | | | | | | | | | | | | | 4 | | | |
| 59 | Tu | Phan | *Tr* | 7.3123° N, 99.8256° E | 30 | 12.v.2019 | | | | 2 | | | | | | | | | | | | | | |
| 60 | Tu | Tharn Pliew | *Sa* | 7.1119° N, 99.8394° E | 107 | 12.v.2019 | | | | 58 | | | | | | | | | | | | | | |
| 61 | Tu | Wang Sai Thong | *Sa* | 7.0909° N, 99.9092° E | 92 | 13.v.2019 | | | | 44 | | | | | | | | | | | 1 | | | |
| 62 | Tu | Ban Suan Tondin | *Sa* | 6.7269° N, 100.1633 °E | 116 | 15.v.2019 | | 23 | | | | | | | | | | | | | | | | |
| 63 | Tu | Pa Tad | *K* | 14.6517° N, 98.7757° E | 161 | 28.x.2019 | 40 | | 3 | | 7 | | | | | | | | | | | | | |
| 64 | Tu | Thi Lo Su (1) | *T* | 15.9274° N, 98.7538° E | 540 | 26.xi.2019 | | | | | | | | | | | | | | | | | | |
| 65 | Tu | Thi Lo Su (2) | *T* | 15.9274° N, 98.7541° E | 549 | 26.xi.2019 | | 3 | | | | | | 1 | | | | | | | | | | |
| 66 | Tu | Pa La Tha | *T* | 15.8191° N, 98.8602° E | 443 | 27.xi.2019 | | 8 | | | | | | | | | | | | | | | | |
| 67 | Tu | Tan Thong | *La* | 19.0699° N, 99.7292° E | | 14.i.2020 | 1 | | | | | | | | | | | | | | | | | 2 |
| 68 | Tu | Wang Kaew | *La* | 19.3164° N, 99.6629° E | 568 | 14.i.2020 | 1 | | | | | | | | | 7 | | | | | | | | |
| 69 | Tu | Pu Kaeng | *CR* | 19.4422° N, 99.6949° E | 540 | 15.i.2020 | 25 | 19 | | 3 | | | | | | 4 | | | | | | | | |
| 70 | Tu | Mae Kae | *La* | 18.7457° N, 99.8164° E | 537 | 16.i.2020 | 31 | 32 | 1 | | | | | | | 6 | | | | | | | | |
| 71 | Tu | Khao Fa | *La* | 18.7496° N, 99.8096° E | 629 | 16.i.2020 | | 1 | | | | | | | | 1 | | | | | | | | |
| 72 | St | Tat Mok | *Ph* | 18.1273° N, 100.2947 °E | 443 | 17.i.2020 | | 11 | | | | | | | | | | | | | | | | |
| 73 | Tu | Huai Rong | *Ph* | 18.4424° N, 100.4495 °E | 450 | 18.i.2020 | 30 | 19 | 1 | | | | | 3 | | | | | | | | | | |

**Table S2.**
Environmental variables at sampling locations. Flow and depth measurements are mean ±SE[n].

| Site code | Conductivity (µS.cm$^{-1}$) | pH | Temperature (°C) | Tree cover (%) | Benthic substrate: cobbles (%) | silt (%) | gravel (%) | sand (%) | rock (%) | mean flow (m.s$^{-1}$) | mean depth (cm) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 90 | | | | | | | 21±2(10-30)[10] |
| 1 | 386 | 8.6 | 25.2 | 100 | | | | | | | 45±9(10-100)[10] |
| 2 | 380 | 8.6 | 24.9 | 100 | | | | | | | |
| 2a | 76.8 | | 24.8 | 10 | | | | | | | 21±3(10-30)[10] |
| 3 | 238 | | 20.5 | 100 | | | 10 | | 90 | | 19±2(10-30)[10] |
| 4 | 86 | | 19.4 | 90 | 30 | 10 | 30 | | 30 | 0.191[1] | 13±2(1-27)[10] |
| 5 | | | | 90 | | | | 10 | 90 | | 11±1(2-21)[10] |
| 6 | 426 | | 25.7 | 100 | | 40 | 10 | | 50 | | 6±1(1-10)[10] |
| 7 | 320 | 8.5 | 24 | 100 | | 40 | 10 | | 50 | | 11±1(0.5-20)[10] |
| 7a | 345 | 8.5 | 23.4 | 100 | | 40 | 10 | | 50 | 0.329±0.048 (0.213-0.580)[10] | 7±2(0.5-15)[10] |
| 7b | 326 | 8.23 | 23.4 | 100 | | 40 | 10 | | 50 | 0.179±0.040 (0.102-0.304[10] | 10±2(0.3-21)[10] |
| 7c | 353 | 8.6 | 22.6 | 100 | | 40 | 10 | | 50 | 0.191±0.054 (0.095-0.288)[10] | 6±1(0.5-20)[10] |
| 7d | 375 | | 26.7 | 100 | | 40 | 10 | | 50 | 0.26±0.49 (0.13-0.36)[10] | 10±2(0.5-20)[10] |
| 8 | 425 | | 25.7 | 45 | 30 | 40 | 10 | 10 | 10 | | 36±8(0.1-80)[10] |
| 9 | 409 | | 25.6 | 90 | | | 5 | 5 | 90 | 0.190±0.008 (0.172-0.205)[11] | 9±1(3-15)[10] |
| 10 | 320 | 8.49 | 23.6 | 90 | | | 5 | 5 | 90 | | 11±2(1-25)[10] |
| 10a | 285 | 8.3 | 28.5 | 90 | | | 5 | 5 | 90 | | 8±2(1-20)[10] |
| 10b | 222 | 8.5 | 24.7 | 90 | | | 5 | 5 | 90 | 0.443±0.032 (0.407-0.553)[10] | 10±3(1-28)[10] |
| 10c | 309 | 0 | 22.2 | 90 | | | 5 | 5 | 90 | 0.2±0.05 (0.15-0.24)[10] | |
| 10d | 410 | | 25.6 | 90 | | | 5 | 5 | 90 | 0.134±0.012 (0.099-0.188)[10] | 9±2(0.5-20)[10] |
| 11 | | | | 70 | | 10 | 50 | 40 | | 0.1[1] | 12±1(6-15)[10] |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| 12 | 642 | | 26.5 | 90 | | | 90 | 10 | | | 14±1(10-20)[10] |
| 13 | 529 | | 27.2 | 50 | | | 25 | 25 | 50 | | 1505±364(10-3200)[12] |
| 14 | 550 | | 27.1 | 50 | | 10 | 80 | 10 | | 0.321±0.115 (0.14-0.85)[10] | 15±3(10-18)[10] |
| 15 | 614 | | 25.6 | 80 | | 10 | 20 | 20 | 50 | | 225±40(52-419)[10] |
| 16 | 492 | | 26.7 | 95 | | 10 | | | 90 | 0.11[1] | 8±1(0.5-13)[10] |
| 17 | 497 | | 27.1 | | | 100 | | | | 0.154±0.047 (0.087-0.213)[10] | 11±2(1-22)[10] |
| 18 | 377 | | 25 | | | 100 | | | | 0.133±0.027 (0.09-0.24)[10] | 10±2(0.5-20)[10] |
| 18a | 432 | | 26.2 | 90 | | | 25 | 25 | 50 | 0.158±0.016 (0.11-0.22)[10] | 46±10(0.5-78)[10] |
| 19 | 786 | 6.9 | 26.2 | 75 | | 80 | | | 20 | | 6±1(0.5-10)[10] |
| 20 | 793 | 6.7 | 25.7 | 90 | | 40 | 20 | 40 | | 0.185±0.016 (0.12-0.28)[10] | 16±3(4-27)[10] |
| 21 | 782 | 6.7 | 27.1 | 75 | | 80 | 10 | | 10 | 0.171±0.024 (0.109-0.25)[9] | 21±4(10-40)[10] |
| 22 | 497 | 8.5 | 25.7 | 100 | 30 | 10 | 30 | | 30 | | 23±5(5-46)[10] |
| 23 | 497 | 8.5 | 25.7 | 100 | 30 | 10 | 30 | | 30 | 0.101±0.007 (0.08-0.12)[10] | 22±4(3-35)[10] |
| 23a | 600 | 7.8 | 27.7 | 90 | | 90 | | 10 | | | 11±2(1-24)[10] |
| 24 | 765 | 7.2 | 25 | 50 | 10 | 5 | 75 | 10 | | 0.155±0.012 (0.12-0.19)[10] | 4±<1(2-6)[10] |
| 25 | 260 | 7.5 | 26.3 | 60 | | | | | | | 2±<1(1-3)[10] |
| 26 | 374 | 9 | 24.4 | 100 | | 5 | 95 | | | | 5±<1(1-8)[10] |
| 27 | 374 | 9 | 24.4 | 100 | | 5 | 95 | | | | 4±1(0.5-7)[10] |
| 27a | 340 | 9.2 | 24.4 | | 10 | 15 | 10 | 15 | 50 | | 478±113(2-1100)[12] |
| 28 | 499 | 9 | 23.2 | 95 | | 10 | 10 | 10 | 70 | 0.204±0.025 (0.14-0.24)[10] | 1501±342(1-2870)[13] |
| 29 | 499 | 9 | 23.2 | 95 | | 10 | 10 | 10 | 70 | 0.187±0.032 (0.15-0.212)[10] | 1540±350(1-3200)[12] |
| 29a | 510 | 8.5 | 23.1 | 100 | 20 | 10 | 50 | | 20 | 0.107±0.018 (0.06-0.19)[10] | 13±2(6-20)[1] |
| 30 | 510 | 8.5 | 23.1 | 100 | 20 | 10 | 50 | | 20 | 0.169±0.025 (0.09-0.23)[10] | |
| 30a | 72 | 8.2 | 20.4 | 100 | 10 | 10 | 70 | 5 | 5 | | 13±3(3-27)[10] |
| 31 | 351 | 9.5 | 21 | 90 | 60 | | 20 | | 20 | 0.291±0.013 (0.2-0.4)[2] | 25±5(1-41)[10] |
| 32 | 660 | 7.4 | 28.6 | 75 | 10 | 10 | 10 | 30 | 40 | | 645±190(1-1590)[10] |
| 33 | 523 | 9.1 | 21.8 | 100 | | 10 | 80 | | 10 | 0.0688±0.177 (0.024-1.27)[10] | 21±4(1-40)[10] |
| 34 | 599 | 9.8 | 24.9 | 100 | | 40 | | 10 | 50 | 0.298±0.038 (0.151-0.536)[12] | 15±2(4-25)[10] |
| 35 | 547 | 9.7 | 23.7 | 45 | | 5 | | 90 | 5 | 0.311±0.062 (0.13-0.42)[10] | 16±3(1-41)[10] |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| 36 | 467 | 8.57 | 24 | 45 | | 5 | | 90 | 5 | 0.219±0.045 (0.14-0.28) [10] | 28±5(4-53)[10] |
| 36a | 705 | 8.4 | 24 | 80 | 30 | | 30 | | 40 | 0.278±0.048 (0.108-0.493)[10] | 31±6(2-57)[10] |
| 37 | 332 | 9.5 | 21.6 | 100 | 10 | | 45 | 10 | 35 | | 28±5(1-39)[10] |
| 38 | 419 | 9.3 | 25.7 | 80 | | 20 | | | 80 | 0.17±0.039 (0.077-0.255)[10] | 1007±310(10-2130)[12] |
| 39 | 423 | 9.7 | 24.6 | 100 | 68 | | 2 | | 30 | | 39±8(10-84)[9] |
| 40 | 445 | 8.2 | 24.8 | 90 | | | 90 | 5 | 5 | | 910±181(20-1750)[10] |
| 41 | 478 | 8.26 | 27 | 60 | 10 | 30 | 10 | | 50 | 0.306±0.024(0.281-0.33)[10] | 13±3(1-25)[10] |
| 42 | 733 | 8.1 | 27.1 | 90 | | 50 | 20 | 20 | 10 | 0.135±0.018 (0.108-0.17)[10] | 4±1(0.5-10)[10] |
| 43 | 591 | 8.11 | 27.7 | 35 | | 20 | | 20 | 60 | | 26±6(2-44)[10] |
| 44 | 246 | 8.7 | 18.6 | 90 | 10 | 10 | 70 | | 10 | 0.132±0.033 (0.099-0.198)[10] | 19±<1(2-3)[5] |
| 45 | 299 | 8.6 | 20 | 60 | | | | | | | 731±208(5-1780)[10] |
| 46 | 39 | 7.7 | 19 | 75 | 40 | 30 | 10 | | 20 | 0.23±0.09 (0.14-0.32)[10] | 35±4(29-45)[10] |
| 47 | 241 | 8.1 | 24.1 | 70 | | | | | | | 19±3(4-37)[10] |
| 48 | 218 | 8.7 | 24.2 | 50 | 90 | | 5 | | 5 | 0.12±0.009 (0.09-0.15)[10] | 17±2(5-35)[10] |
| 49 | 210 | 8.7 | 24.8 | 95 | 50 | | 20 | 20 | 10 | 0.113±0.011 (0.06-0.14)[10] | 19±5(2-49)[10] |
| 50 | 201 | 8.6 | 28.1 | 30 | 10 | 10 | 60 | 10 | 10 | 0.181±0.025 (0.09-0.26)[10] | 903±240(0.5-2100)[10] |
| 51 | 157 | 8.6 | 26.8 | 50 | 30 | 5 | 50 | 10 | 5 | 0.277±0.015 (0.24-0.31)[12] | 534±150(1-1050)[10] |
| 52 | 206 | 8.5 | 24 | 95 | 10 | 10 | 60 | 10 | 10 | 0.221±0.055 ( 0.06-0.31)[10] | 18±4(0.2-45)[10] |
| 53 | 366 | 8.38 | 22.2 | 70 | | 80 | | 5 | 15 | 0.131±0.022 ( 0.108-0.154)[2] | 6±1(1-10)[10] |
| 54 | 293 | 8.7 | 23.7 | 95 | 50 | 5 | 12.5 | 12.5 | 20 | 0.18±0.026 (0.117-0.270)[10] | 1146±1(1-2680)[10] |
| 55 | 305 | 8.36 | 27.1 | 90 | | 30 | | | 70 | | 1621±283(1-3000)[10] |
| 56 | 227 | 7.27 | 28.5 | 70 | | 5 | 90 | 5 | | | 495±209(1-1400)[10] |
| 57 | 331 | 8.4 | 27.2 | 80 | 30 | 30 | | 25 | 15 | 0.174±0.044 (0.099-0.033)[10] | 562±202(1-1600)[10] |
| 58 | 274 | 8.2 | 26.5 | 90 | 10 | 60 | 5 | 10 | 15 | | 535±191(1-1540)[10] |
| 59 | 265 | 8.47 | 26.2 | 100 | 20 | 30 | | | 50 | | 725±158(0.2-2700)[10] |
| 60 | 290 | 8.6 | 25.7 | | | 20 | 20 | | 60 | | 1451±582(0.1-5000)[11] |
| 61 | 407 | 8.3 | 25.7 | 70 | | 80 | | | 20 | | 4±1(0.3-11)[10] |
| 62 | | | | 100 | | 20 | 20 | 10 | 50 | 0.19±0.065 (0.044-0.544)[10] | 789±319(1-3400)[10] |
| 63 | 365 | 8.5 | 22.4 | 80 | | | | 80 | 20 | | 9±3(1-26)[10] |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 64 | 365 | 8.4 | 22.4 | 100 | | 70 | 15 | 10 | 5 | 0.195±0.022 (0.117-0.248)[10] | 10±3(0.5-29)[10] |
| 65 | 312 | 8.55 | 23.1 | 90 | 10 | 10 | | | 80 | 0.248±0.023 ( 0.147-0.359)[10] | 227±220(1-2240)[10] |
| 66 | 301 | 8.5 | 22.5 | | | 10 | | | 90 | 0.182±0.028 (0.063-0.233)[10] | 618±207(0.5-2100)[10] |
| 67 | 200 | 8.4 | 19.8 | 60 | | 30 | | | 70 | 0.108±0.034 (0.044-0.199)[8] | 734±293(0.1-2550)[10] |
| 68 | 228 | 8.05 | 20.4 | 100 | 10 | 25 | 10 | 5 | 50 | 0.251±0.036 (0.177-0.372)[10] | 661±250(0.8-1960)[10] |
| 69 | 271 | 8.6 | 20.9 | 100 | | | | | | | 11±4(0.2-33)[10] |
| 70 | 307 | 8.3 | 21.9 | 85 | | 30 | | | 70 | 0.199±0.045 (0.044-0.466)[10] | |
| 71 | 264 | 8.8 | 20.4 | 100 | 40 | | 10 | | 50 | | |
| 72 | 326 | 8.5 | 22 | 100 | | 10 | 10 | | 80 | | |
| 73 | | | | | | | | | | | |

**Table 3.**
Environmental parameters at sites where *Hemerodromia* species were found. Abundance (A) and number of sites where a taxon was present ($N_s$) are indicated. Conductivity, pH and temperature are shown as mean ± SE (range) [n] and the proportion of occurrences of each species near tufa formations ($P_t$), fast films ($P_{ff}$) and waterfalls ($P_w$) is indicated.
Means of samples ($log_{10}$ transformed abundance data) sharing the same lower case letters *a*, *b*, *c* and *d* were significantly different from each other ($P<0.05$) in ANOVA using Turkey's pairwise test. Samples indicated by *X* ($P<0.001$) or *Y* ($P<0.05$) had ranges significantly different from the population mean (based on unit SD >3.29 standard deviations). Statistical tests used $log_{10}$ transformed data for only the 10 commonest species with abundances >1% of total).

| **Species** | **A** | **$N_s$** | **Conductivity (µS.cm$^{-1}$)** | **pH** | **Temperature (°C)** | **$P_t$** | **$P_{ff}$** | **$P_w$** |
|---|---|---|---|---|---|---|---|---|
| *Hcon* | 597 | 16 | 369±21 (200–576) [24] *a* | 8.6±0.1 (8.05–9.7) [19] | 23.7±0.9 (19.8–28.5) [25] *Y* | 1 [26] | 1 [26] | 1 [23] |
| *Hfus* | 504 | 47 | 396±25 (10–793) [54] *a,b,X* | 8.4±0.1 (4.9–9.8) [46] *X* | 24.6±0.3 (18.6–28.6) [54] *X* | 0.72 [54] | 0.57 [54] | 0.53 [51] |
| *Hano* | 139 | 12 | 371±17 (271–499) [17] *c* | 8.7±0.1 (8.2–9.5) [14] | 24.0±0.5 (20.9–28.5) [17] | 1 [20] | 0.9 [20] | 0.83 [18] |
| *Hfur* | 116 | 6 | 203±42 (39–365) [8] *b,c,d* | 8.5±0.2 (7.7–9.6) [7] | 23.1±1.1 (19–26.5) [8] | 0.66 [6] | 0.66 [6] | 0 [5] |
| *Hyun* | 85 | 21 | 464±38 (39–793) [21] *d,X* | 8.4±0.2 (6.7–9.5) [17] *X* | 24.4±0.6 (18.6–28.6) [21] *X* | 0.69 [23] | 0.5 [22] | 0.52 [23] |
| *Hnam* | 77 | 4 | 357±16 (285–426) [9] *X* | 8.4±0.1 (8.2–8.5) [5] | 25.2±0.6 (23.4–28.5) [9] *X* | 1 [11] | 1 [11] | 1 [11] |
| *Hdem* | 38 | 7 | 239±26 (157–366) [7] *Y* | 8.5±0.1 (8.1–8.7) [7] *Y* | 24.8±0.8 (22.2–28.1) [7] *a* | 0.43 [7] | 0.43 [7] | 0.57 [7] |
| *Hzet* | 33 | 7 | 291±24 (201–365) [7] *Y* | 8.7±0.1 (8.4–9.5) [7] | 23.7±0.8 (21.6–28.1) [7] | 0.71 [7] | 0.57 [7] | 0.71 [7] |
| *Hacu* | 24 | 4 | 249±75 (39–374) [4] | 8.7±0.3 (7.7–9.2) [4] | 21.6±1.6 (19–24.4) [4] *a,Y* | 0.5 [4] | 0 [4] | 0 [4] |
| *Hore* | 23 | 6 | 294±33 (200–423) [6] | 8.6±0.2 (8.1–9.7) [6] | 21.9±0.8 (19.8–24.6) [6] *X* | 1 [6] | 1 [6] | 0.75 [4] |
| *Hani* | 9 | 2 | 214±4 (210–218) [2] | 8.7±0 (8.7–8.7) [2] | 24.5±0.3 (24.2–24.8) [2] | 0.5 [2] | 0.5 [1] | 0.5 [2] |
| *Hmen* | 8 | 1 | 157±0 (157–157) [1] | 8.6±0 (8.6–8.6) [1] | 26.8±0 (26.8–26.8) [1] | 0 [1] | 0 [1] | 0 [1] |
| *Hali* | 7 | 2 | 214±4 (210–218) [2] | 8.7±0 (8.7–8.7) [2] | 24.5±0.3 (24.2–24.8) [2] | 0.5 [2] | 0.5 [2] | 0.5 [2] |
| *H. dep* | 5 | 1 | 423±0 (423–423) [1] | 9.7±0 (9.7–9.7) [1] | 24.6±0 (24.6–24.6) [1] | 1 [1] | 1 [1] | 0 [1] |
| *Hsam* | 5 | 2 | 310±20 (290–331) [2] | 8.5±0.09 (8.4–8.6) [2] | 26.45±0.75 (25.7–27.2) [2] | 1 [2] | 1 [2] | 0.5 [2] |
| *Hfla* | 3 | 1 | 86±0 (86–86) [1] | | 19.4±0 (19.4–19.4) [1] | 0 [1] | 0 [1] | 0 [1] |
| *Hpai* | 2 | 1 | 201±0 (201–201) [1] | 8.6±0 (8.6–8.6) [1] | 28.1±0 (28.1–28.1) [1] | 0 [1] | 0 [1] | 0 [1] |

**Table 4.**

Monthly variation of diversity and abundance of *Hemerodromia* species at all sites at latitudes ≥14°N. Species richness (*S*) = number of species; Relative abundance (*A**) =S/E where E= the sampling effort (number of days).

| | Month | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Jan** | **Feb** | **Mar** | **Apr** | **May** | **Jun** | **Jul** | **Aug** | **Sep** | **Oct** | **Nov** | **Dec** |
| **Species richness (*S*)** | 6 | 1 | 9 | 9 | 3 | 4 | | | 4 | 7 | 6 | 5 |
| **Relative abundance (*A**)** | 28.1 | 9.5 | 53.8 | 35.0 | 29.6 | 68.0 | | | 14.0 | 25.4 | 14.0 | 28.8 |
| **Effort (*E*)** | 8 | 2 | 4 | 7 | 5 | 1 | 0 | 0 | 4 | 10 | 13 | 5 |

| **Lineage** | **N (no. haplotype)** | **Haplotype diversity** | **Nucleotide diversity** | **Tajima' D** | **Fu's Fs** |
|---|---|---|---|---|---|
| A (Loei) | 114 (5) | 0.0692±0.0330 | 0.00013±0.00027 | -1.89042* | -6.95357 * |
| B (Lampang + Chiang Rai) | 56 (16) | 0.7721 ± 0.0398 | 0.00323 ± 0.00203 | -1.32235 | -6.20787* |
| C (Phrae) | 25 (8) | 0.8333 ± 0.0441 | 0.00319 ± 0.00205 | 0.37724 | -1.52753 |
| D (Tak + Kanchanaburi) | 64 (14) | 0.4390 ± 0.0790 | 0.00134 ± 0.00116 | -2.18118* | -14.79528* |
| E (Chiang Mai) | 3 (2) | 0.6667 ± 0.3143 | < 0.0001 | N/A | N/A |
| F (Mae Hong Son) | 1 (1) | N/A | N/A | N/A | N/A |
| **All** | **263 (46)** | **0.7820 ± 0.0218** | **0.02276 ± 0.01146** | **0.95806** | **-3.46909** |

Table 5. Genetic diversity indices of genetically divergent lineages of *Hemerodromia conspecta* in Thailand (P<0.05 is indicated by*).

**10. REPOT APPENDIX**

This work was generously funded by the Thailand Research Fund (DBG6180024) who are acknowledged with sincere gratitude

## Publications:

The following papers have been published. –

**Plant AR** (2019) Biomediation of tufa formation by silk capture nets of larval Philopotamidae (Insecta: Trichoptera). *Tropical Natural History*, **19**, 113–116.

**Plant AR** (2020) New species of *Hemerodromia* Meigen (Diptera: Empididae: Hemerodromiinae) associated with limestone karstic waters in Thailand. *Zootaxa* **4738**, 549–560.

The following papers have been submitted for publication (both to *Entomological Science*). –

**Kunprom C, Plant AR, Pramual P** (submitted 2020) Population genetic structure and microendemism in aquatic Empididae (Diptera) in transient tufa biotopes on tropical karst.

**Plant AR** (submitted 2020) Tropical tufa and karsts streams support unique and threatened assemblages of aquatic Diptera, Empididae in Thailand.

The following informal article has been published. –

**Plant, AR** 2019 Inventory of aquatic Hemerodromiinae and Clinocerinae (Diptera: Empididae) inhabiting tufa stream environments of tropical karst ecosystems in Thailand. *Fly Times*, **62**, 5–7.