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### Two new subspecies of Hemicordulia tenera Lieftinck, 1930 (Corduliidae) from Cambodia and Thailand

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### Abstract

*Hemicordulia tenera donnellyi* ssp. nov. (holotype  $\sigma$ : Chieng Mai Prov., Kunklang: highway 1009, Restaurant; 16°32.0' N 98°31.3' E, 1000 m, 22 v 1996, FSCA) and *H. t. vikhrevi* ssp. nov. (holotype  $\sigma$ : Cambodia, Koh Kong Province, ~13 km ENE of Koh Kong, '*Hemicordulia* brook', 11°39'55'' N, 103°05'34'' E, 315 m, 04 xii 2010, RMNH) are described from North Thailand and South-West Cambodia, respectively. The nominotypical *H. tenera tenera* Lieftinck, 1930 is distributed in the Malay Peninsula, Borneo, Java and Sumatra. Although these three subspecies are genetically very close, they are distinguishable by the relative length and shape of the caudal appendages.

**Key words:** dragonfly, Odonata, Anisoptera, Corduliidae, *Hemicordulia tenera*, *donnellyi* ssp. nov., *vikhrevi* ssp. nov., new subspecies, Thailand, Cambodia

### Introduction

*Hemicordulia* is a large genus of Corduliidae dragonflies consisting of 39 species (Steinmann 1997; Tsuda 2000; Dijkstra 2007). It attains its maximum diversity in Australia and New Guinea inhabited by nine species each. It also includes many endemic species living in islands belonging to 'Wallacea' (Sulawesi, Flores, Buru, Sumba), Melanesia (the Solomons, New Caledonia, Norfolk, Vanuatu, Fiji), Micronesia (the Mariannas, Guam, Palau) and Polynesia (New Zealand, Kermadec, Samoa, Thaiti etc.) (Steinmann 1997; Asahina 1947, 1980, 1987; Tsuda 2000). Several species are found west and north of the Wallace line, in Sundaland (the area used to be united into the palaeo-continent 'Sunda' during the ocean level drops in the Pleistocene) and further north and west, where they allopatrically produced few young vicariant species. *H. australiae* 

(Rambur, 1842) has a limited penetration just to Bali, H. tenera Lieftinck, 1930 has a broad distribution over Java, Sumatra, Borneo, and Malay Peninsula including the peninsular Thailand (= H. gracillima Fraser, 1944, see Steinmann 1997 and http:// zipcodezoo. com/Animals/H/Hemicordulia\_tenera/ revised by Van Tol J., 07 iii 2006), H. apoensis Asahina, 1980 is an endemic of the Philippines, H. mindana Needham & Gyger, 1937 ranges from Philippines to Guam and Taiwan, and also in south parts of Japan (the subspecies H. m. nipponica Asahina, 1980). Both H. ogasawarensis Oguma, 1913 and H. okinawensis Asahina, 1947 are Japanese endemic species with narrow distribution areas. Hemicordulia asiatica has separate distribution areas in India, Ceylon, Myanmar, and its spread westwards over the Indian Ocean produced a number of very similar neoendemic species on its islands and Africa: H. africana Dijkstra, 2007, H. atrovirens Dijkstra, 2007, H. similis (Rambur, 1842), H. virens (Rambur, 1842) (Dijkstra 2007). Recently H. edai Karube & Katatani, 2012 was described from Laos as closely related to *H. asiatica* (Karube & Katatani 2012); and an unidentified but also related to *H. asiatica*, most probably the same species, was reported from Huizhou, Guandong Province of China (Wu 2012).

In 1994, Prof. Thomas W. ['Nick'] Donnely (1994: 86) reported the specimens of "*Hemicordulia* undescr. sp." from Doi Inthanon National Park Headquarters, Chaing Mai Province, Thailand, which "is similar to *tenera* but has a different appendage and is larger". However, later he changed his opinion so that the Doi Inthanon specimens are conspecific to *H. tenera* Lieftinck, 1930 (pers. comm.). Hämäläinen & Pinratana (1999) reported *H. tenera* for Loei Province of Thailand, but still attributed the specimens from Chiang Mai Province to *Hemicordulia* sp. Prof. T.W. Donnelly kindly borrowed us three of his specimens, and Brother Amnuay Pinratana kindly sent a male from Loei Province.

In December 2010, OEK collected in Koh Kong Province of Thailand three male specimens of *Hemicordulia* identical to *H. tenera* in all respects but with relatively shorter caudal appendages; they were referred to as *Hemicordulia* sp. in (Kosterin 2011, 2012a, b). In August 2012, OEK revisited Cambodia and collected 11 more male specimens in Koh Kong and Kampot Provinces (Kosterin 2012a, b). Using these specimens, we reconsidered the taxonomic status of continental specimens related to *H. tenera*.

Species of *Hemicordulia*, as well as the closely related genus *Procordulia* Martin, 1907 are often very close to each other. Moreover, both genera are broadly distributed over oceanic islands, and isolated populations with unclear taxonomic status are found, that makes their taxonomy complicated and appealing for a revision (van Tol 1997; Marinov 2012). On the other hand, minor but permanent differences in the shape of appendages and vulvar lamina exhibited by populations of some Indian Ocean islands and East Africa were considered to be diagnostic at the species level (Dijkstra 2007). Here we propose two new subspecies of *H. tenera* from N Thailand and SW Cambodia, respectively. Although the relative length of the caudal appendages did not overlap in them, so that the differences are is comparable in magnitude to the differences of a species level, e.g. proposed by Dijkstra (2007) for African species, no clear genetic differences were observed.

## Methods

Specimens from SW Cambodia were collected during the survey of the Odonata fauna of that country supported by International Dragonfly Fund. Their original labels include conventional names given by OEK to nameless brooks and rivulets for simplicity (Kosterin 2011, 2012a, b), given in single quotations; details of the provenance of the type specimens not given in labels are added below in square brackets.

Three of the four specimens collected at Doi Inthanon, N Thailand, were borrowed from Thomas W. Donnelly; one of the four specimens collected in Loei Province, NE Thailand, was received from Brother Amnuay Pinratana.

The specimens were examined in the laboratory using a stereomicroscope; the lengths of leg segments and pterostigma were measured by ocular-micrometer. Illustrations of morphological details were prepared using lens Zeiss Stemi 2000-C with digital camera Canon PowerShot A640 at the Institute of Molecular and Cellular Biology of Siberian Branch of Russian Academy of Sciences, Novosibirsk. Images with broad focus zones were obtained from serial photos with shifted focus using Helicon Focus 5.1 program (http://www.photo-soft.ru/heliconfocus.html).

The epiproct length related to the length of the caudal appendages was measured as the proportion P = E / C where C is the length of the caudal appendages as measured in the lateral view from its apex to the point where the lower margin of the caudal appendages meets the epiproct upper margin and E is the epiproct length as measured from its apex to the same point, as shown in Figure 1. Because the epiproct apex is not always seen from above, we measured C and E from the lateral view.



Figure 1. A scheme of measuring the cercus length, C, and the epiproct length, E, against the lateral view of the appendages of the paratype of *Hemicordulia tenera donnellyi* ssp. nov. from Phu Kradung, Loei Province, Thailand.

Nuclear DNA sequences of ITS1 and ITS2 and mitochondrial DNA sequences of mt16SrRNA and mtCOI were obtained from one paratype of *H. tenera vikhrevi* ssp. nov., as described below, and from two specimens of *H. tenera tenera* from Malaysia and Indonesia, as well as from representatives of some other corduliid species.

One leg of each dried specimen was homogenized and DNA was extracted using QIAamp DNA mini Kit (Qiagen, Japan). The relevant DNA fragments were amplified by polymerase chain reaction (PCR) with the following primers: 5'-GGC CAA ACT TGA TCA TTT AG-3' and 5'-GCC GGC CCT CAG CCA G-3' for ITS1 (Futahashi & Sasamoto 2012), 5'- CGG TGG ATC ACT CGG CTC GT-3' and 5'- TTT CAC TCG CCG TTA CTA AGG GAA TC-3' for ITS2 (Futahashi & Sasamoto 2012), 5'- CCG GTC TGA ACT CAG ATC ACG CTG TTT ATC AAA AAC AT-3' for mt16SrRNA (Palumbi et al., 1991), 5'- ATA ATT GGR GGR TTY GGR AAY TG-3' and 5'- CCA AAR AAT CAA AAT AAR TGT TG-3' for mtCOI (Hayashi et al. 2005). PCR products were treated with exonuclease I and shrimp alkaline phosphatase, and sequenced directly by an ABI3130xl genetic analyzer (Applied Biosystems, USA). The sequence data were deposited in the DDBJ/EMBL/GenBank databases (accession numbers are shown in Figure 7).

Sequences were aligned using Clustal\_X (Thompson et al. 1997). Molecular phylogenetic analyses were conducted by three methods, neighbor-joining with MEGA6 (Tamura et al. 2013), maximum likelihood with MEGA6 (Tamura et al. 2013), and Bayesian with MrBayes v3.1.2 (Ronquist & Huelsenbeck 2003). Maximum Composite Likelihood model (pairwise deletion) and Tamura Nei model (use all sites) were used for neighbor-joining and maximum likelihood analysis, respectively (Tamura & Nei 1993). Bootstrap values for neighbor-joining and maximum likelihood analysis, respectively (Tamura & Nei 1993). Bootstrap values for neighbor-joining and maximum likelihood phylogenies were obtained by 1,000 bootstrap replications. For Bayesian analysis, Markov chain Monte Carlo (MCMC) analysis was performed under the General Time Reversible + Invariable sites (GTR + I) model. The burn-in period was determined graphically. In total 3750 trees were generated for each Bayesian analysis (ngen = 500,000, sample-freq = 100, burn in = 1,250).

### Abbreviations

- FSCA Florida State Collection of Arthropods, Division of Plant Industry, Grainesville, Florida, USA;
- ISEA Institute of Systematics and Ecology of Animals, Siberian Branch of Russian Academy of Sciences, Novosibirsk, Russia;
- RMNH Naturalis Biodiversity Centre, Leiden, the Netherlands;
- ZMUM Zoological Museum of Moscow State University, Russia.

### Hemicordulia tenera vikhrevi ssp. nov.

(Figures 2, 3)

Hemicordulia sp. - Kosterin 2011: 99-100 Hemicordulia undescr. spec. - Kosterin 2012a: 78 Hemicordulia sp. - Kosterin 2012b: 81-83 Hemicordulia sp. - Kosterin et al. 2012: 162 Hemicordulia tenera ssp. - Kosterin 2014: 34

### Etymology

The subspecies is named in honour of Dr. Nikita Vikhrev, a Russian dipterologist from Moscow, who first observed this dragonfly in the type locality on 03 xii 2012 and informed the first author about it.

#### Specimens examined

Holotype o' (Figure 2a-f): Cambodia, Koh Kong Province [the Cardamom Mts southwest foothills], ~13 km ENE of Koh Kong, '*Hemicordulia* brook', 11°39'55'' N, 103°05'34'' E, 315 m a.s.l., 15 viii 2011, O.E. Kosterin. Deposited in RMNH.

#### Paratypes

The same place as the holotype, 04 xii 2010, O.E. Kosterin - 3  $\sigma\sigma$  (Figures 2g, h; 3a-d). Of them, 1  $\sigma$  depo-sited in the Kanagawa Prefectural Museum of Natural History, Odawara, Kanagawa, Japan, this paratype was involved into molecular analysis; 2  $\sigma\sigma$  in ZMUM. Cambodia [Koh Kong Province, the Cardamom Mts south-west foothills], 16 km ENE of Koh Kong, '*Nannophya* rivulet', 11°40′14-22″ N, 103°07′32-38″ E, 292-295 m a.s.l., 16 viii 2011, O.E. Kosterin - 6  $\sigma\sigma$  (Figures 2i, j; 3e-l), deposited in RMNH, ISEA and ZMUM. Cambodia, Kampot Pro-vince, Bokor Plateau, 3.6 km NE Bokor Place, 'Odorata' sandy road, 10°39′03-13″ N, 104°02′14-26′ E, 921-927 m a.s.l., 18 viii 2011, O.E. Kosterin - 2  $\sigma\sigma$  (Figures 3m, n), 1 of them depo-sited in RMNH, the other is kept with OEK, to be later deposited at ISEA.

#### Diagnosis

Very close to *H. tenera tenera* Lieftinck, 1930 but differing in the cerci less processed apically and so relatively shorter as compared to the epiproct, yet not so short and arched as in *H. tenera donnellyi* ssp. nov.; the ratio of the epiproct length to the length of caudal appendages in lateral view is 0.85-0.90.

#### Description of the holotype, male

An elegant large-headed, slender and quite hairy dark-coloured dragonfly (Figure 2a).

**Head** large, densely set with rather short hairs which are dark on frons, vertex and occiput, brownish to almost white in lower face part whitish behind eyes (Figure 2b), but the rest of rear surface naked. Labium whitish-grey becoming chrome yellow at

margins of movable parts. Labrum dull chrome yellow. Clypeus and sides of frons dull olive brownish, with anteclypeus darker and postclypeus posterior margin chrome yellow. Fore side of frons flat, bright chrome yellow, above becoming shining emerald green through a reddish-brown transitory zone; frons upper side convex with a central groove broadening to its posterior margin. Vertex shining emerald green, very convex,



Figure 2. The holotype of *Hemicordulia tenera vikhrevi* ssp. nov.: habitus (a) and morphological details (b-f), and paratypes (g-j). - a, general habitus at capture; - b, thorax and head rear side; - c, right wings; - d, appendages, lateral view; - e, appendages, dorsal view; - f-h, hamuli; - i, penis, vesica seminalis, dorsal view; - j, vesica seminalis, lateral view. - (g, h) paratypes from the type locality (04 xii 2010) and (i, j) paratype from the '*Nannophya* rivulet' (16 viii 2011).

subtriangular in frontal view. Occipital triangle reddish-brown, set with brown hairs and with a fringe of lighter hairs at margin, alike those around eyes. Rear side glossy black. Eyes dark-grey with an emerald-green lustre when alive.

Thorax. Prothorax light brown with scarce short hairs. Synthorax (Figure 2b) quite densely set with yellowish-brown hairs, longer and lighter on mesepisternum. Pterothorax light brown with some greenish lustre and with the following parts emerald metallic green (which has very indistinct borders): mesepisternum except for its lower part, mesepimeron except for its central area, metepisternum at its anterior margin below spiracle and in dorsoposterior corner, anterior part of metepimeron; antealar ridges dark, margined with light brown at mesothorax, light brown in metathorax; space between wings brown.

Legs long, all coxae light-brown, very hairy; protrochanter light brown, mesotrochanter brown, metatrochanther brownisgh-black; profemur light brown but blackish at anterior side and distal end, other femora blackish-brown; all tibia black, protibium with a brown inner keel for distal 0.8 of its length; metatibium with a brown inner keel for about its entire length; tarsi black, claws brownish-black.

Wings (Figure 2c) hyaline with a faint ochraceous-yellow wash in hind wing proximal half gradually disappearing in its distal half and hardly noticeable in fore wing basal half; membranule greyish brown. Fore wing with 7 antenodals and 5 postnodals; hind wing with 5 antenodals and 6 postnodals. Arculus oblique, situated between antenodals 1 and 2; triangle 2-celled on fore wing, entire in hind wing; hypertriangles entire; fore wing subtriangle 3-celled; cubital space with a cross-vein; anal triangle entire. Anal loop 9-celled but its posterioproximal border indistinct as formed by angled inner borders of two last cells. Pterostigma short, dark brown.

Abdomen slender, at S1-2 slightly swollen, in lateral view S7-9 a bit higher than others (S9 slightly extending down), in dorsal view S3-4 slightly narrower, otherwise almost cylindrical (Figure 2a). S2 with long brownish-yellow hairs at proximal and lower margins and dorsal side; S8-9 with medium-long dark hairs fringing tergite lower margins, S10 terminus with dense and rather long hairs; otherwise abdomen in a very fine hardly noticeable pubescence, becoming longer at dorsal side of S7-10. Tergites black, moderately glossy, with a slight purple to greenish lustre; lower and central parts of S1-2 and proximal part of S3 dark-brown; S4-7 with hardly seen very diffuse dark-brown lateral spots at tergite lateral sides starting at about 1/8 and ending at about 5/8 of their length; these spots are better expressed at tergite ventral sides; on S3-4 their inner margins enhanced with light-brown.

Anal appendages (Figure 2d, e) slender, cerci very dark brownish black, epiproct dark brown. Cerci about 1.28 times as long as S9+S10. In dorsal view, they are slightly S-like curved, converging to apices or even crossed at tips, rather constricted soon at base, then gradually broadening to middle and moderately tapering to rather blunt, non-processed apices. In lateral view they are evenly arched, rather evenly narrow, rounded at apices; in distal 1/3 inner margin of the caudal appendages is seen since this part of the caudal appendages is somewhat turned inwards along its axis.

**Epiproct** in ventral view very broad at base then immediately narrows and then gradually tapering to apex, in dorsal view mostly obscured by cerci; in lateral view it is inversely arched towards cerci, being slightly more curved at distal part than at proximal one, with a blunt apex directed almost vertically. The P value, being a measure of the epiproct relative length (see methods), is 1.00.

Ventral side of S2 set with hairs exceeding hamuli height in length. Secondary genitalia grown. Genital lobe mammiform, set with long hairs. Hamulus posterioris broad at base, then processing to a long thick smoothly arched hook gradually narrowing in distal half but slightly inflated and rather blunt apex (Figure 2 f; Paratypes 2g, h).

Vesica seminalis (Figure 2 i, j dissected in a paratype): light brown with a dark brown glans. Vesicle with a pair of lateral rounded tubercles at apex; segment 2 with a dorsal hook at ca distal 1/3, segment 3 hood-shaped, glans with two rounded sclerotised lobes, membranous part projecting dorsally, flagellae long and twisted.

**Measurements** [mm]. Hind wing 30, abdomen without appendages 30, body (with head and appendages) 45. Profemur (without trochanther) 4.5, protibium 4.7, meso-femur 5.5, mesotibium 5.3, metafemur 6.0, metatibium 5.9. Caudal appendages 2.7. Pterostigma 2.0 in forewing, 1.7 in hind wing.

#### Variation in paratypes

The three males obtained in December 2010 at the '*Hemicordulia* brook' (the type locality) do not exhibit yellowish spots on the abdomen which seem to be obscured by darkening associated with ageing (Kosterin 2011). The same concerns two of seven males collected in Koh Kong Province in August 2011. Other specimens have the spots varying from obscure dark yellowish to very obscure dark-brown, most frequently seen on S4-8. The ochraceous wash of the wing membrane varying from slight and expressed in the hind wing basal half only (5 of 8 specimens from '*Nannophya* rivulet' of August 2011) through appearing over the entire membrane but very faint beyond the hind wing basal half (2 specimens from Bokor) to quite well expressed all over the wings (1 specimen from '*Nannophya* rivulet' and 1 specimen from '*Hemicordulia* brook' of August 2011). General hairiness of the thorax seems to vary as well, that may reflect the age of an insect. The abdomen length varies from 29 to 31 mm, the hind wing length from 28 to 30 mm. No variation detected in the number of antenodals and postnodals; the number of cells in the anal loop varies from 9 to 10.

Variation of the appendages in the paratypes is shown in Figure 3. The inner margin of the caudal appendages is seen from lateral view in the distal 1/2-1/3 of the caudal appendages. The P value, being a measure of the epiproct relative length (see methods), is 0.90 and 0.90 in the two paratypes from the type locality of December 2010, 0.86, 0.88, 0.88, 0.89, 0.89 and 0.90 in the paratypes from the '*Nannophia* rivulet', 0.85 and 0.89 in the two paratypes from Bokor Plateau.

Female unknown.

#### Habitat and behaviour

In Cambodia, *H. tenera vikhrevi* ssp. nov. was first observed and collected on 03-04 xii 2010 on gentle coastal foothills of the Cardamom Mts covered with lower hill forest, at a clear '*Hemicordulia* brook' flowing through pools of variable depth along the border of the forest and a recently cleared area with some small plantation. Three individuals patrolled, by restless flying and hovering up to 1 m above water surface, respectively a 3 x 8 m pool ca 1.5 m deep with clear banks, a shallower pool with overgrown banks (both are shown in fig. 14 in Kosterin 2011) and a very shallow pool with a noticeable current. All they were 'hyperadults' with the abdomen darkened up to complete invisibility of yellow spots. On 15 viii 2011 two individuals were observed at the same brook, and the next day, 16 viii 2011 many males were observed hovering over reaches of the '*Nannophia* rivulet' km NE of the previous locality (Kosterin 2012a, for the locality image see fig. 18 in Kosterin 2011), from where they were absent in xii 2010, and also over the similar '*Macromia* rivulet' km further NE from where it was not collected (see fig. 59b in Kosterin 2012a). In the first half of the day the males



Figure 3. Anal appendages of paratypes of *Hemicordulia tenera vikhrevi* ssp. nov. in lateral (a, c, e, g, i-n) and dorsal (b, d, f) view. - a-d, paratypes from the type locality of 04 xii 2010; - e-l, paratypes from the '*Nannophya* rivulet' of 16 viii 2011; - m-n, paratypes from Bokor Plateau of 18 viii 2011.

occupied, shared and patrolled all reaches of the 'Nannophia rivulet' with sandy or silty bottom, either shady or exposed, either deep and slow or fast, but obviously avoided rapidous sections with rocky bottom. Sometimes they were observed chasing each other; capturing a male lead to appearing of another one at the same reach some 10-15 minutes later. Around noon the males disappeared from exposed reaches but remained at shady ones. A flying copula was observed at an exposed reach, which landed onto a small bush, making pulsing movements around once a second, and disjoined after a while.

Thus, in August the species appeared more numerous and widespread in that area than in December, and generally showed a low selectivity as to its lotic habitats. The August specimens showed variable expression of the yellow spots of the abdomen hence being younger on average. Most probably, imagines of *H. tenera vikhrevi* ssp. nov. are on the wing round the year but are more numerous in summer. All the three mentioned localities in Koh Kong Province were situated at 290-315 m a.s.l.

The other habitat of the new subspecies was situated atop the Bokor Plateau at 921-932 m a.s.l., in guite a different environment: a foggy plateau covered with upland hill and low montane forest and partly by open savannah-like areas (loc. veal) on acidic soils and Sphagnum mires. Again, there was an uncertain sighting on 09 xii 2010 (Kosterin 2011) while numerous individuals were found on 18-19 viii 2011 (Kosterin 2012a, b), that is the species has the same annual pattern of abundance on the plateau as at the lower levels in Koh Kong Province. On sunny 19 viii 2011, two males were observed patrolling, since 11:00 am, half-shaded reaches with slow to moderate currents and sandy beds of the Popokvil River (see fig. 57 in Kosterin 2012a and fig. 9 in Kosterin 2012b). In contrast to crystal clear brooks and rivulets in Koh Kong Province, this river, flowing from Sphagnum mires, had brown water and carried guite a lot of yellowish foam as rich in humic acids. Besides, a male was quite unexpectedly found patrolling a small forest swamplet close to the river, with sedge tussocks leaving no open water (see fig. 37b in Kosterin 2012a). In the same area, at 1:00-1:20 pm on 18 viii 2011, at overcast weather between rains, about five patrolling males appeared over the shallowest pools with sandy bottoms and over the water, flowing over a sandy road crossing a large veal (see fig. 58 in Kosterin 2012a) with a sedgy small brook near by. This appeared some unusual outburst, for they were absent from this seemingly unsuitable locality before, at 11:50-12:10 am, and after 3:30 pm, with the weather varying from short sun through overcast to drizzling rain, as well as throughout the next sunny day.

We may conclude that *H. tenera vikhrevi* ssp. nov. inhabits a variety of lotic habitats in forested foothills and mountains and tolerates some anthropic disturbance of its habitats.

#### Distribution

The Cardamom Mts in a broad sense (including the Damrei or Elephant Mts) in Cambodia; known from Koh Kong and Kampot Provinces. Expected for eastern Thailand and Phu Quoc Island of Vietnam.

#### Hemicordulia tenera donnellyi ssp. nov.

Figures 4, 5

Hemicordulia undescr. sp. - Donnelly, 1994: 84 Hemicordulia sp. - Hämäläinen & Pinratana, 1999: 91 Hemicordulia tenera (Lieftinck, 1930), part. - Hämäläinen & Pinratana, 1999: 90

#### Etymology

The subspecies is named in honour of Professor Emeritus Thomas W. Donnelly, an American geologist and outstanding odonatologist, who first collected and recognised the new subspecies.

#### Specimens examined

**Holotype** ° (Figures 4, 5a, e, f): Chieng Mai Prov., Kunklang: highway 1009, Restaurant; 16°32.0' N 98°31.3' E, 1000 m a.s.l., 22 v 1996: 96x012; in FSCA collection.

#### Paratypes

Thailand, Chieng Mai Prov., Kunklang: highway 1009, Restaurant; 16°32.0' N 98°31.3' E, 1000 m a.s.l., 22 v 1996: 96x012 - 1  $\sigma$  (Figure 5b, g); in RMNH collection. Thailand, Chieng Mai Prov., pond at Doi Inthanon N.P. headquarters [18°32.16' N 98°31.43' E], 1249 m a.s.l., 15-16 vii 1994, coll. & det. [as *H. tenera*] T. W. Donnelly: 94x061. - 1  $\sigma$ (Figure 5c, h). Thailand, Loei Province, Phu Kradung, 13 v 1995 - 1  $\sigma$  (Figure 5d), deposited in the Kanagawa Prefectural Museum of Natural History, Odawara, Japan.

#### Additional, not studied specimens

Thailand, Chieng Mai Prov., pond at Doi Inthanon N.P. headquarters, 15-16 vii 1994, coll. & det. T. W. Donnelly (the same as the holotype) - 3 °°, collection by T. W. Donnelly. Thailand, Loei Province, Phu Kradung, 13 v 1995 - 3 °° in coll. Brother Anmuay Pinratana, Bangkok, Thailand.

#### Diagnosis

Very close to *H. tenera tenera* Lieftinck, 1930 and *H. tenera vikhrevi* ssp. nov. but differing from both in the further shorter cerci with blunt apices, not or very slightly extending beyond the epiproct apex (the ratio of the epiproct length to the length of the caudal appendages in lateral view is 0.92-1.00), their distal 1/3 noticeably bent downward and look somewhat inflated in lateral view.

#### Description of the holotype, male

General habitus similar to the above subspecies but with the yellow marking more developed, perhaps because of the specimens being young (Figure 4a, b).

**Head** large, densely set with rather short hairs which are dark on frons, vertex and occiput, brownish to almost white in lower face part whitish behind eyes. Labium whitish-grey more saturated chrome yellow at anterior margins of movable parts.

Labrum dull chrome yellow. Fore side of frons flat, bright chrome-red, above becoming shining emerald green through a reddish-brown transitory zone; frons upper side convex with a central groove broadening and flattening to posterior margin. Vertex shining violet-green, very convex, subtriangular in frontal view. Occipital triangle dark-brown, set with light hairs. Rear side glossy black.



Figure 4. Holotype of *Hemicordulia tenera donnellyi* ssp. nov. - a, habitus; - b, head and thorax; - c, accessory genitalia.

Thorax. Prothorax light brown with scarce short hairs. Synthorax (Figure 4b) quite densely set with yellowish-brown hairs, longer and lighter on mesepisternum. Pterothorax brownish-yellow with the following emerald metallic green areas: mesepisternum except for its lower part (to half of its length at dorsal suture); on mesepimeron: an area at its anterior margin broadening ventrally to occupy its ventral part and ascend along its posterior margin to spiracle level; on metepisternum: very ventroanterior corner, a narrow area at dorsoposterior corner and along posterior margin at upper part; on metepimeron: anterior part. Antealar ridges only somewhat darker brown than synthorax background.

Legs long, all coxae light-brown, prothrochanter light brown, mesotrochanter bicolorous, with brownish black anterior side and light-brown posterior side, metatrochanter brownisgh-black; profemur light brown but blackish at anterior side and distal end, mesofemur blackish-brown with brown lightening of posterior side of its proximal part, metafemora blackish-brown; all tibia black, protibium with a brown inner keel for distal 0.8 of its length; metatibium with a brown inner keel for about its entire length; tarsi black, claws brownish-black.

Wings (Figure 4a) hyaline with a faint ochraceous-yellow wash more expressed in hind wing proximal half; membranule brownish-greyish; neuration brown to dark brown, its numerical parameters as in the previous subspecies. Pterostigma short, dark brown.

Abdomen (Figure 4a) shape as in the subsequent subspecies. S1 and dorsal and ventral sides of S2 with long brownish-yellow hairs; S7-9 with shorter dark hairs fringing tergite lower margins, S10 terminus with long hairs; otherwise abdomen with a very fine pubescence, longer at dorsal side of S7-10. S1 muddy brown, S1 yellowish with dorsal side dark with some metallic lustre. Other tergites black with a greenish metallic lustre at dorsal and lateral sides but with pairs of large dark-yellow lateral spots: in S3 spot occupies its entire lower half, nearly so in S4 but spot ends rounded not reaching segment apex, with some addition expansion of yellow along lateral seam; spot on S5 narrower and occupying 3/4 of its length; those on S6-S8 further narrower and occupy about 1/3 of their length. Tergite ventral side yellowish on S3-4 entirely and on S5-S8 for the same length as spots above.

Anal appendages (Figure 5a, e, f) dark brownish black, epiproct somewhat slighter. Cerci about 0.80 times as long as S9+S10. In dorsal view, they are slightly S-like curved, constricted soon at base, broadening substantially at middle and very slightly tapering to blunt apices. They are noticeably bent down at 2/3 of their length, then somewhat turned inwards along their axes so that inner margin is seen from lateral view making cerci looking as if slightly inflated since that point. Cerci apices rounded, a level with epiproct apex. Epiproct as in previous subspecies. The P value, being a measure of the epiproct relative length (see methods), is 1.00.

Ventral side of S2 set with hairs exceeding hamuli height in length. Secondary genitalia yellowish but hamular hook dark brown; their shape as in the previous subspecies.

Vesica seminalis not examined because the specimens were acetonised.

**Measurements** [mm]. Hind wing 30, abdomen without appendages 30, body (with head and appendages) 46. Profemur (without trochanther) 4.8, protibium 5.0, mesofemur 6.4, mesotibium 6.2, metafemur 8.4, metatibium 8.1. Caudal appendages 2.4. Pterostigma 2.0 in forewing, 1.8 in hind wing.



Figure 5. Anal appendages of *Hemicordulia tenera donnellyi* ssp. nov. in lateral (a-d), ventral (e) and dorsal (f-h) views: a, e, f - holotype; b, g - paratype with the same label; c, h - paratype from Doi Inthanon National Park headquarters; d - paratype from Phu Kradung National Park, Loei Province.

### Variation in paratypes

The two paratypes from Chiang Mai Province are very similar to the holotype on the whole. The Loei Province paratype has the yellowish-brown pattern darker and abdominal spots smaller that may reflect its older age. The abdomen and hind wing length in the paratypes is 31-32 mm; total body length 46-47 mm. The paratype from the type locality has 8 rather than 7 antenodals on the fore wing and 7 rather than 6 postnodals on the hind wing. The Loei paratype has 7 antenodals on the fore wing and 5 antenodals on the hind wing and an 8-celled anal loop.

The P value, being a measure of the epiproct relative length (see methods), is 0.93 in both paratypes from Chiang Mai Province (Figure 5b, c, g, h) and 0.92 in the paratype from Loei Province (Figure 5d).

### Female unknown.

### Habitat

The specimens of the new subspecies were collected at a pond (1994). The specimens were taken in Chiang Mai Province in May and July, in Loei Province in May.

### Distribution

Northern Thailand: known from Chiang Mai and Loei Provinces. Expected for southern Laos.

## Remarks

The main difference among *H. tenera vikhrevi* ssp. nov., *H. tenera donnellyi* ssp. nov., and *H. tenera tenera* is the length and shape of the caudal appendages. In *H. tenera donnellyi* ssp. nov., it is blunter than in *H. tenera vikhrevi* ssp. nov., noticeably bent down, and somewhat inflated at 2/3 of its length in lateral view. In *H. tenera vikhrevi* ssp. nov., the lateral view of the caudal appendages is evenly and slightly arched and in most specimens look evenly thick throughout its length. In both new subspecies, the caudal appendages are not so processed and curved inwards at tips compared to *H. tenera vikhrevi* ssp. nov., 0.92-1.00 in *H. tenera donnellyi* ssp. nov., and 0.76-0.81 in *H. tenera tenera* (Figure 6; also see Lieftinck 1930: fig. 22; Asahina 1987: figs 63-64; Tang et al. 2010: 124), and larger than any other *Hemicordulia* species living in west and north of the Wallace line; 0.79 in *H. asiatica* (Dijkstra, 2007), 0.76 in *H. edai* (Karube & Katatani, 2012), 0.78 in *H. mindana* (Asahina, 1947), 0.79 in *H. nipponica*, 0.77 in *H. okinawensis*, and 0.78 in *H. ogasawarensis* (Asahina, 1947).

Notably, the length of caudal appendages changes geographically; the southernmost *H. tenera tenera* (living in 08°S - 06°N) has the longest caudal appendages, *H. tenera vikhrevi* ssp. nov. (living in 10.5-11.5°N) has a medium long caudal appendages, and *H. tenera donnellyi* ssp. nov. (living in 19°N) has the shortest caudal appendages.

Prof. T. W. Donnelly kindly informed us that both curvature and relative length of any cylindrical structure of an insect depends on the haemolymph pressure during eclosion when the structure is spreading before cuticular hardening. Variation in the relative length and curvature could be caused by differences in this pressure, which may depend on some environmental factors rather than genetic differences. This may be validated by rearing larvae in the same condition.

*H. tenera vikhrevi* ssp. nov. also differs from both *H. tenera tenera* and *H. tenera donnellyi* ssp. nov. in darker and smaller brownish-yellow pattern elements, although this difference may be due to age differences; greater age of the Cambodian specimens collected in August-December, while the Thai specimens were collected in May-July. No size difference is recognized among the three subspecies. Unfortunately, females of two new subspecies have not been collected.

At present, the distribution area of these three subspecies is split by lowlands. The mountain along the Burmese Fault (habitats of *H. tenera tenera* and *H. tenera donnellyi* ssp. nov.) is mostly composed of igneous rocks, whereas mountains of the Cardamoms (habitat of *H. tenera vikhrevi* ssp. nov.) is composed of gentle sandstone.

One paratype of *H. tenera vikhrevi* ssp. nov. was subjected to molecular analysis as well as two specimens of *H. tenera tenera* and several other corduliid species. Nuclear DNA sequences from ITS1 to ITS2, and two mitochondrial DNA sequences, 16SrRNA, and COI were analysed. All three specimens of *H. tenera* spp. were extremely close genetically, and no phylogenetic relationships between them could be revealed (Figure 7), suggesting that they are conspecific. Based on the clear differences of caudal appendages and their distribution areas, we propose here that they belong to different subspecies.



Figure 6. Holotype of *Hemicordulia tenera tenera*. Lieftinck, 1930. - a, general habitus; - b, envelope; - c-e, appendages in lateral (c), dorsal (d) and ventral (e) view.



Figure 7. Molecular phylogenetic analysis of *Hemicordulia tenera* spp. and some other Corduliidae based on nuclear genes ITS1-5.8S-ITS2, 722 bp (a) and mitochondrial genes 16SrRNA and COI, 961 bp (b). A neighbor-joining phylogeny is shown, while maximum likelihood and Bayesian phylogenies exhibited substantially similar topologies. On each node, statistical support values are indicated in the order of [bootstrap value of neighbor-joining]/[bootstrap value of maximum likelihood]/ [posterior probability (percentages) of Bayesian]. Asterisks indicate support values lower than 50%. *Hemicordulia tenera vikhrevi* ssp. nov. is represented by a paratype from Cambodia, Koh Kong Province, ~13 km ENE of Koh Kong, 'Hemicordulia brook', 11°39'55'' N, 103°05'34'' E, 315 m a.s.l., 04 xii 2010, deposited in the Kanagawa Prefectural Museum of Natural History, Odawara, Kanagawa, Japan.

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