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Aeshna vercanica sp. nov. from Iran with a new insight into the *Aeshna cyanea*-group (Odonata: Aeshnidae)

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Abstract. *Aeshna vercanica* sp. nov. is described and illustrated. The male holotype and four male paratypes were collected on 15-vii-2013 in the Hyrcanian forest of the Alborz Mountains, Māzandarān province, northwestern Iran. A specimen collected on 29-vi-2002 in the Talysh Hills, Lankoran area, Azerbaijan, also belongs to the new species. In July 2014 the species, including females, was recorded again at the type locality and additionally ca 400 km further east in Golestān province. Males are similar to *Aeshna cyanea* in the structure of genitalia and terminalia but differ in head morphology, pterostigma length, colour pattern, and behaviour. Females have small abdominal blue or turquoise postero-median dorsal spots which are absent on S9 and S10, thin green antehumeral stripes, a less robust appearance than females of *A. cyanea*, and are more slender and longer. The range of *A. vercanica* sp. nov. covers the Hyrcanian forest along the southern margin of the Caspian Sea. Analysis of the barcoding COI sequence of DNA confirmed that *A. vercanica* sp. nov. is separated from *A. cyanea* by a genetic distance of ca 4 %. The ITS gave a similar result. A haplotype map could not derive *A. vercanica* sp. nov. directly from *A. cyanea*. They are thus related but different species, and we suggest the common ancestor lived in pre-Pleistocene times. Analysis of *A. cyanea* specimens from across its range also revealed a western clade from the Maghreb to Central Europe. Populations from the Caucasus to Eastern Europe were polytomous, a common scenario for post-glacial invaders. A molecular comparison of the species pair *A. juncea* and *A. subarctica* showed these to be even more closely related than *A. cyanea* and *A. vercanica* sp. nov.

Key words. Dragonfly, Anisoptera, new species, Alborz Mountains, Caspian Sea, Hyrcanian forest

Introduction

Five species of the genus *Aeshna* have previously been recorded from Iran (Heidari & Dumont 2002; Rastegar et al. 2013). Among these, *Aeshna*

cyanea (Müller, 1764) is known from a single male only, collected over 100 years ago (Ris 1930; Schmidt 1954). More than a decade ago, a male, classified as a »blue point mutation« of *A. cyanea* was collected on 29-vi-2002 in the Talysh Hills, Lankaran area, Azerbaijan, close to the Iranian border (Dumont 2004). It patrolled a pathway in dark forest (HJD unpubl.)

In 2013, ES, JS, and TS discovered an unusual *cyanea*-like *Aeshna* in the Hyrcanian forest of the Alborz Mountains in northwestern Iran. One year later, in July 2014, this taxon was recorded again at the same sites. In this study we describe this *Aeshna* as a new species and analyse its relationship with *A. cyanea*.

Material and methods

Field work

Field trips were carried out by ES, JS, and TS in July 2013, and by TS and Dietmar Ikemeyer in July 2014. The region visited was the Hyrcanian forest of the Alborz Mountains in northwestern Iran, Māzandarān province, north of the village of Veysar.

On 15-vii-2013, an unusual *cyanea*-like *Aeshna* was observed in the Hyrcanian forest on a forest trail from Veysar to Musa-Abad (36°35'02"N, 51°31'57"E; Fig. 1), at about 460 m a.s.l., in the evening at 18:30 h IRDT (Iran Daylight Time; UTC + 4:30 h).

Male individuals were netted. Additional males and one female were captured at the same locality one year later. In July 2014 we visited Golestān province, about 400 km east of the type locality. Here, near the village of Loveh, a second female was collected. All specimens were kept in acetone for 12 h, then dried. Measurements were taken in mm and are given as mean and range. Photographs of live male and female animals were taken in the field and details were photographed upon return to Europe.

Measurements and statistics

Some characteristics of size, head morphology, colours, and the number of cells in the anal loop and the discoidal triangle of the hind wing of the new species were compared with 103 males of *Aeshna cyanea* from large parts of its enormous distribution area, including Europe, the Urals, and the Caucasus region (Tab. 1). The length of cerci relative to the last two abdominal

segments of the two females of the new species were compared to females of *A. cyanea* from Europe and the Caucasus region (n = 31). In the latter case no statistics were used as only two females of the new species were available. All animals used for analysis had been conserved by comparable methods. Differences in the morphological characters of males were evaluated using a Mann-Whitney-U-Test and the complete sample size is shown as Tukey boxplots with the extremes.

Molecular analysis

Full details of the methods and calculations used can be found in Guan et al. (2013) and Dumont et al. (2010). A summary is given here. DNA was extracted from a leg of three specimens of the new species from Māzandarān, Azerbaijan, and Golestān, and from 19 specimens of *A. cyanea* from dif-

Figure 1. Hyrcanian Forest where *Aeshna vercanica* sp. nov. was observed hunting (type locality). 12 km north of Veysar village, Māzandarān province, Iran (15-vii--2013); Photo: ES

Table 1. Provenance and collectors of the specimens of *Aeshna cyanea* and *A. vercanica* sp. nov. used for statistical analysis. All specimens were fixed in acetone. NHMB – Natural History Museum Berlin.

Provenance	Collection, collector
A. vercanica sp. nov. (n = 14)	
Azerbaijan, Lankaran (1)	H.J. Dumont
Iran, Golestan (5)	E., J. & T. Schneider
Iran, Māzandarān (8)	E., J. & T. Schneider
A. cyanea (n = 103)	
Armenia, Lori Province (1)	V. Ananian
Armenia, Tavush Province (3)	V. Ananian
Austria, Salzburg (4)	NHMB, G. Peters
Belgium, Affligem (2)	H.J. Dumont
Finland, Espoo (1)	E., J. & T. Schneider
France, Aude (1)	E., J. & T. Schneider
France, Bretagne (14)	NHMB, G. Peters
Germany, Brandenburg (6)	E., J. & T. Schneider
Germany, Brandenburg (25)	NHMB, G. Peters
Germany, Hamburg (1)	E., J. & T. Schneider
Germany, Hesse (3)	E., J. & T. Schneider
Germany, Saxony-Anhalt (1)	E., J. & T. Schneider
Germany, Saarland (1)	E., J. & T. Schneider
Germany, Thuringia (5)	NHMB, H. Hackethal
Italy, Sicily (1)	E., J. & T. Schneider
Poland, West Pomerania (2)	E., J. & T. Schneider
Russia, Komi (1)	NHMB, G. Peters
Russia, Moscow (15)	NHMB, G. Peters
Russia, Mostovsky, Caucasus (2)	S. Ferreira
Sweden, Smarland (14)	NHMB, G. Peters

ferent localities across its range, including outliers in the Maghreb and the Caucasus region (Tab. 2), using a procedure modified from those previously described (Dumont et al. 2007; Xu et al. 2011). A 1 mm section of a leg was cut off with a scalpel and transferred to a tube with 20 µl 0,05N NaOH and 2 µl 5 % Tween 20. This was heated for 15 minutes at 95°C and cooled

Table 2. Provenance and collectors of the specimens of *Aeshna cyanea* and *A. vercanica* sp. nov. and other species of *Aeshna* used for molecular analysis. NHMB – Natural History Museum Berlin.

Provenance	Collection, collector, details
A. vercanica sp. nov. (n = 3)	
Azerbaijan, Lankaran (1)	H.J. Dumont
Iran, Golestan (1)	E., J. & T. Schneider
Iran, Māzandarān (1)	E., J. & T. Schneider
A. cyanea (n = 19)	
Algeria (1)	B. Samraoui
Armenia, Lori Province (1)	V. Ananian
Armenia, Tavush Province (3)	V. Ananian
Belgium (1)	H.J. Dumont
Georgia (1)	M. Seehausen
Germany, Baden-Württemberg (1)	B. Kunz
Germany, Berlin (1)	E., J. & T. Schneider
Italy (1)	A. Cordero Rivera
Poland, West Pomerania (2)	E., J. & T. Schneider
Russia, Mostovsky, Caucasus (4)	S. Ferreira
Spain (1)	A. Cordero Rivera
Tunisia (2)	M. Korbaa
A. grandis (n = 1)	
Germany, Brandenburg (1)	E., J. & T. Schneider
A. juncea (n = 2)	
Germany, Brandenburg (1)	E., J. & T. Schneider
GenBank (1) (Japan, The Netherlands)	ITS: AB706686, 714 bp, DNA linear INV 15-JUN-2012 COI: KF369278, 658 bp, DNA linear INV 03-NOV-2013
A. subarctica (n = 1)	
Germany, Brandenburg (1)	E., J. & T. Schneider
A. viridis (n = 2)	
Germany, Brandenburg (2)	E., J. & T. Schneider

on ice. 100 µl sterile water was added to the tube and mixed. 1 or 2 µl was used in a PCR reaction. We amplified and sequenced a fragment of mt DNA (the barcoding segment of the cytochrome c oxidase subunit I (COI) gene)

in the Iranian specimen and the entire region separating the SSU and LSU region of the 18S nuclear rDNA operon comprising the ITS 1 intergenic spacer, the conserved 5.8S gene, and the ITS 2, using the polymerase chain reaction (PCR) on a 2720 Thermal Cycler of Applied Biosystems, CA, USA. Primers used for PCR were CO1490F and CO2198R (Folmer et al. 1994) for the COI fragment. Primers used for amplifying the rDNA fragment are listed in Dumont et al. (2007). The PCR products were sequenced on an ABI 3130XL automatic sequencer of Applied Biosystems. The analysis of the fragments, after alignment, consisted of estimating the phylogenetic tree of the *cyanea*-like animals of different origin (Bayesian post-hoc probability), and calculating the genetic distance (Kimura 2-parameter value) between the sequences for the Iranian species and the related *A. cyanea.* parameter value (K2P). Gaps were treated as a fifth character. The outgroup consisted of the *Aeshna juncea*-group (*A. juncea* and *A. subarctica*), *A. viridis*, *Anax*, and *Boyeria*. For the latter two, sequences were taken from GenBank. A haplotype map was constructed from the COI information, using the program TCS v.1.21, with standard settings (Clement et al. 2000).

***Aeshna vercanica* sp. nov.**

(Figs 2–6, 9a, 10a–c, 11a, 13a–d, 14a)

Material studied

Holotype ♂. Iran, Māzandarān province, forest trail ca 12 km north of Veysar village (36°35'02"N, 51°31'57"E; elevation ca 460 m a.s.l.;), 15-vii--2013, leg. TS; to be deposited in Museum für Naturkunde Berlin, Germany (ZMHB).

Paratypes. 4♂, same data as holotype; 3♂, 1♀, same site as holotype, 12-vii--2014, leg. TS, all in coll. E.J.T. Schneider; 5♂, 1♀, Iran, eastern Golestān province, shaded mountain rivulet with cascades near Loveh village (37°20'39"N, 55°39'51"E; elevation 598 m a.s.l.), 20-vii-2014, leg. TS, in coll. E.J.T. Schneider; 1♂**,** Azerbaijan, Talysh Hills, Rivo village ca 30 km west of Lankaran city, 29-vi-2002, leg. HJD (cf. Dumont 2004), in coll. H.J. Dumont.

Etymology

The specific epithet refers to the unique Caspian Hyrcanian mixed forest. The adjective *vercanica* is the latinised form of Old Persian *Verkâna* ("Wolf-

land"). Hyrcania, the contemporary name for the region south and south-east of the Caspian Sea in historic accounts is based on the Greek borrowing (Υρκανία) of the Old Persian name.

Male (holotype)
Head (Fig. 9a) – Anteclypeus, postclypeus and labrum largely pale blue to turquoise, upper ventral edges of mandibula turquoise, frons massive and angular, without a visible T-mark but with a triangular depression. Dorsum as well as vertex dark to dark purple, occipital triangle very small and black, with the triangle base >0.8 mm. Basis of antennae pale silverish, antennae black, compound eyes large and coloured deep blue in live animals broadly touching; head comparatively massive in dorsal view.

Thorax – Prothorax black, mesepisternum black, mesoinfraepisternum and metainfraepisternum yellow, front of synthorax black with narrow, basally narrowing small turquoise antehumeral stripes, not occupying more than 20 % of the ante-humeral region. Sides of synthorax with two broad turquoise spots separated by a continuous broad black bar that fully encloses the metastigma (Fig. 10a). Legs black.

Wings – Hyaline. Apices densely veined, leading edge dark brown to black, anal triangle with 3 cells, pterostigma black, hind wing with 5 cells in the discoidal triangle and 16 cells in the anal loop (Fig. 5).

Abdomen – Constricted at base, black with a pair of shining blue postero-median dorsal spots on S1–10. S1 black, laterally pale turquoise and with small blue dorsal spots, S2 with a pair of larger blue postero-median spots and additionally a pair of thin pale turquoise line markings, laterally two larger blue markings, auricle triangular, with a black base and a black margin, the central area blue, 5 denticles on dorsal side of each auricle, male accessory genitalia as in Figure 4. S3 constricted, with a pair of larger blue postero-median spots and additionally a pair of thin pale turquoise lines, the basal lateral part has a larger blue marking. S4–10 each with a pair of blue postero-median spots, very small in S10, the lateral blue markings becoming smaller from S3 to S7. S8–10 without blue lateral spots, without spots

on the ventral side, and with small dorsal spines at the hind margins of each tergite (Fig. 11a). Upper appendages paddle-shaped, expanding towards the end, and abruptly narrow to form a down-turned spine, like an eagle's head seen laterally; upper appendage is slightly longer than S9 and S10.

Measurements [mm] – Hw length 49; Hw width 15; pterostigma on hw 3.6; upper appendage 5.6; lower appendage 2.9.

Variation in Paratypes

On the frons of two of the 14 males is an obcure T-shaped marking with a very thick and short stem. The basally narrowing small ante-humeral stripes and the two spots on the sides of the synthorax are turquoise in the holotype but can be blue in other specimens. Only the specimen from Azerbaijan has a very small narrow yellow-green slit at the metastigma (Fig. 10a) and another specimen a barely visible, blue, very faint and short line (Fig. 10b); for variation see Figs 10a–c.

Figure 2. Perching male of *Aeshna vercanica* sp. nov., ca 12 km north of Veysar village, Māzandarān province, Iran (12-vii-2014). Photo: Dietmar Ikemeyer

Figure 3. Pinned male specimen of *Aeshna vercanica* sp. nov., collected ca 12 km north of Veysar village, Māzandarān province, Iran (14-vii-2013), in lateral view. Photo: Ekkehard Wachmann

Figure 4. *Aeshna vercanica* sp. nov., male accessory genitalia of the holotype. Photo: Ekkehard Wachmann

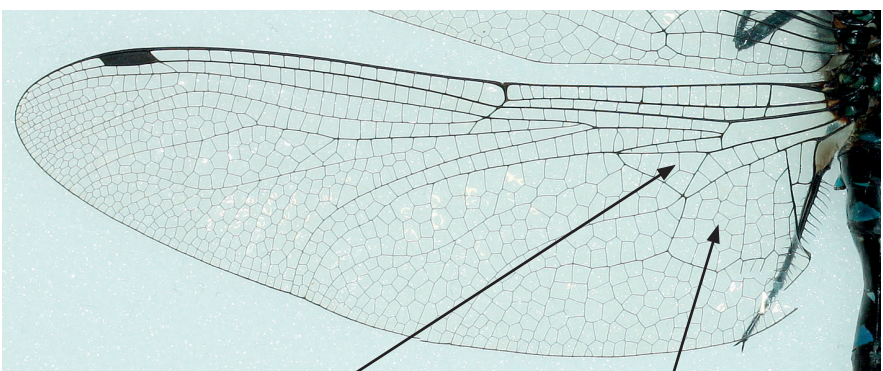

Figure 5. *Aeshna vercanica* sp. nov., hind wing of male holotype with 5 cells in the discoidal triangle and 16 cells in the anal loop. Photo: Ekkehard Wachmann

Female

Head (Fig. 13a) – Anteclypeus, postclypeus, and labrum largely pale green, upper ventral edges of mandibula yellow-green, frons massive and angular yellow, a clear T-mark with a thick stem, dorsum as well as vertex dark to dark black, occipital triangle small and black, basis of antennae pale silverish, antennae black, compound eyes large and coloured deep blue with broad median connecting zone; head comparatively massive.

Thorax – Prothorax black, mesepisternum black, mesoinfraepisternum, and metainfraepisternum green, front of synthorax black with green, basally narrowing, very small ante-humeral stripes, not occupying more than 10 % of the ante-humeral region (Fig. 13a). Sides of synthorax with two broad

Figure 6. Female of *Aeshna vercanica* sp. nov., ca. 12 km north of Veysar village, Māzandarān province, Iran (12-vii-2014). Note the small blue spots, the slender body and the long cerci. Photo: Dietmar Ikemeyer.

green stripes separated by a continuously broad black bar that fully encloses the metastigma. Legs black except for reddish-brown femora.

Wings – Hyaline. Apices densely veined, leading edge dark brown to black (Fig. 13b), pterostigma black (Fig. 13c), hind wing with 4 cells in the discoidal triangle and 16 cells in the anal loop.

Abdomen – Slender, black with a pair of shining blue postero-median dorsal spots on S1–6. S1 black, laterally green and with blue dorsal spots, S2 with a pair of larger blue postero-median spots and additionally a pair of thin pale blue line markings and laterally two larger green markings. S3 with a pair of larger blue postero-median spots and additionally a pair of thin blue lines, the basal lateral part has a larger green marking, S4–6 with a pair of blue postero-median spots, becoming smaller which each segment to the end and missing on the last two segments, and lateral green markings that are becoming smaller from S3 to S6. S7–10 without green lateral spots, no spots or markings on S9 and 10. Cerci long and thin paddle-shaped, longer than the last two segments (Fig. 13d). Hind margin of basal plate deeply incised, two plates of valvulae 3 half of the length in parallel (Fig. 14a).

Measurements [mm] – Hw length 49; Hw width 16; pterostigma length on Hw 3.5; length of cerci 4.9.

General diagnosis

A conspicuous aeshnid with dark overall appearance, blue compact eyes, synthorax marked turquoise or blue with continuous, broad dark stripe. Abdomen with a pair of shining blue postero-median spots on each segment. Females have similar small abdominal blue spots, very thin and small green ante-humeral stripes and a slender abdomen with long cerci.

Molecular analysis and genotypical comparison

A phylogenetic tree based on the COI fragment, using Bayesian inference, is shown in Figure 7. The ITS tree revealed similar results. The K2P genetic distance was 4.5 % for COI; for ITS it was 6.8 %. The ITS of the Iranian and Azeiri specimens was identical.

Analysis of the COI of 19 *A. cyanea* specimens from different localities across its wide range placed eastern animals (Russian Caucasus, Armenia, and Georgia) in polytomy, while in Western and Southern Europe, including Italy and North Africa, a subclade occurs. The populations in Tunisia and Algeria are recovered as relicts, containing several private haplotypes. *Aeshna vercanica* sp. nov. is well separated from *A. cyanea* specimens of all regions including the geographically nearest *A. cyanea* populations in

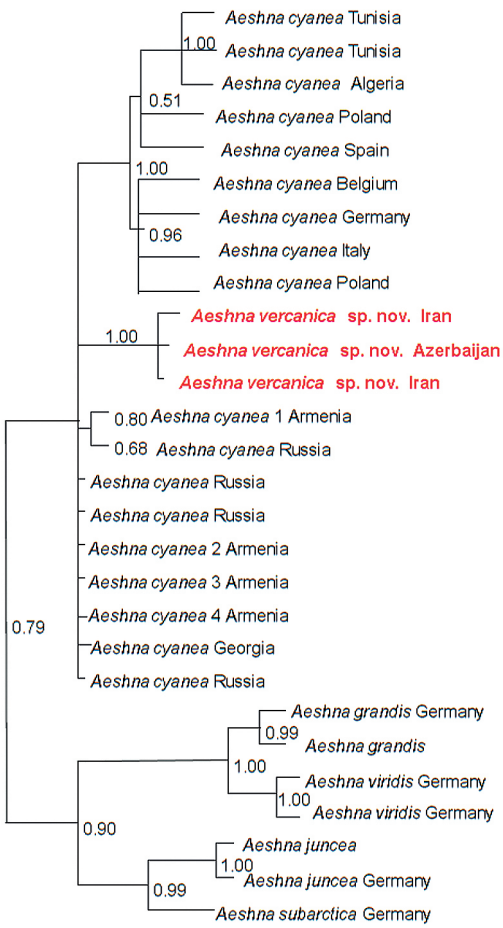

Figure 7. *Aeshna* tree showing the relationship between *Aeshna cyanea* from different origins and *Aeshna vercanica* sp. nov., *A. juncea*, *A. subarctica*, and *A. viridis*. *Aeshna vercanica* sp. nov. is recovered as a subclade of the *A. cyanea* complex.

the Armenian provinces Tavush and Lori (Fig. 7). *Aeshna viridis* is recovered as only remotely related to *A. cyanea* and turns out to be a relative to *A. grandis. Aeshna juncea*, and *A. subarctica* are closely related and are in fact closer than *A. cyanea* and *A. vercanica* sp. nov. (Fig. 7).

The haplotype map shows that, under standard settings, the TCS program could not directly link *A. vercanica* sp. nov. to *A. cyanea*, suggesting a long evolution in isolation (Fig. 8).

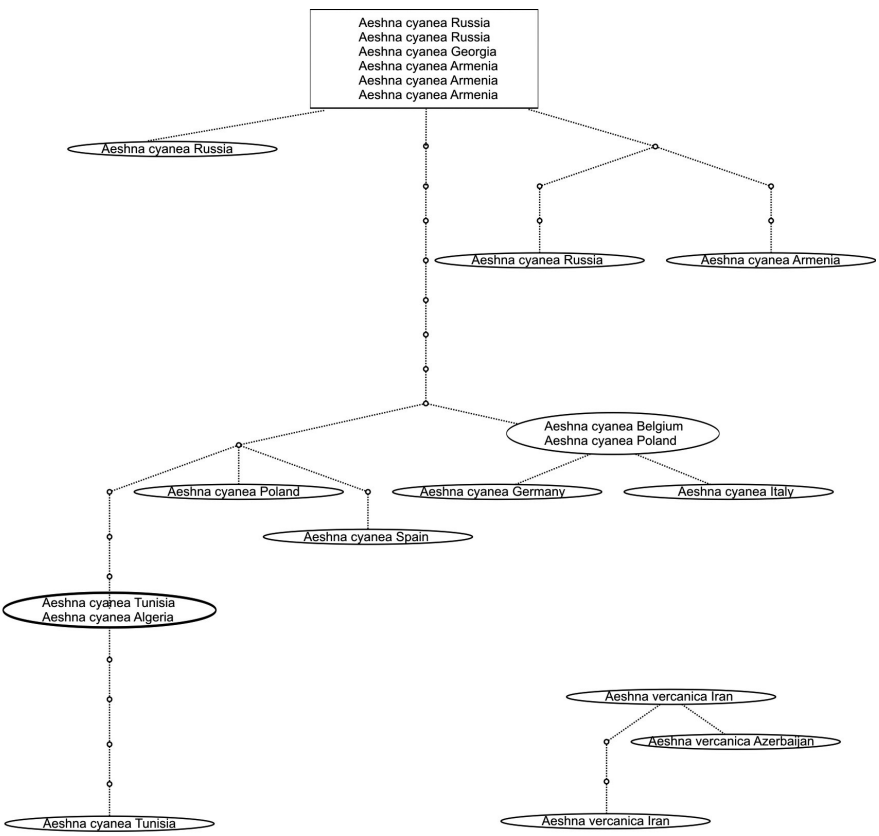

Figure 8. Haplotype map (TCS program, standard settings) of *Aeshna vercanica* sp. nov. in relation to *A. cyanea.*

Table 3. Diagnostic characters separating *Aeshna vercanica* sp. nov. from *A. cyanea*

Character	*A. vercanica* sp. nov	*A. cyanea*
Frons in males (Fig. 9)	A T-shaped marking is usually not visible due to the black background; if visible, T with thick stem and broad base	With T-shaped marking on yellow background; T with thin stem and without broad base
Frons in females (Fig. 13)	T-shaped marking with thick, short stem and with broad base	T-shaped marking with thin stem and without broad base
Frons+vertex/confluence of the eyes in males	<1	>1
Occipital triangle base in males (Fig. 9)	<1 mm	>1,2 mm
Dark band laterally on synthorax in both sexes (Fig. 10)	Continuous dark band, enclosing the metastigma; a small, narrow blue-green slit may be present	Asymmetric fork left and right of the metastigma
Ante-humeral stripes in both sexes	Narrower, turquoise or blue	Wider, green
Abdominal colour pattern in both sexes	Small shining dark blue spots on S1-S7	Large green spots on S1-S7 in adults (in tenerals sometimes light blue)
Ventral abdominal spots	Absent	Present
Dorsum of S9 + 10 in males	With separate blue spots	With blue band
Spots on S9 + 10 in females	absent	present usually as blue band
Pterostigma length in males (Fig. 12)	>3.5 mm	<3.2 mm
Small dorsal spines on hind margins of S8-10 (Fig. 11)	Present	Absent
Pterostigma colour in females (Fig. 13)	Black	Reddish-brown
Wing venation in females (Fig. 13)	Fore edge dark brownish-black	Fore edge reddish-brown
Female appendices (Fig. 13)	> than the last two segments	< than the last two segments
Ovipositor (Fig. 14)	hind border of basal plate deeply incised, two plates of valvulae 3 half of the length in parallel	hind border of basal plate less incised, two plates of valvulae 3 less than half of the length in parallel

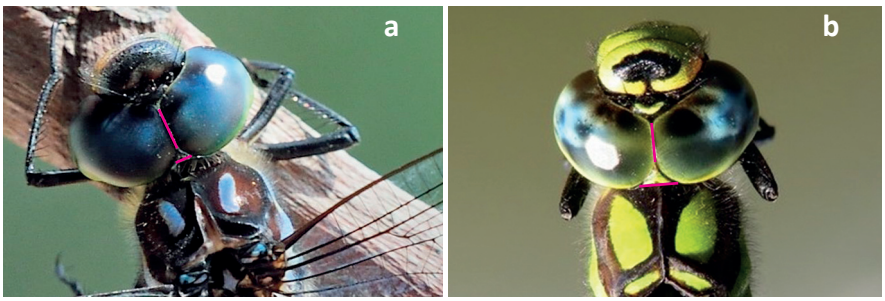

Figure 9. Heads of male *Aeshna vercanica* sp. nov. from Iran (9a) and *A. cyanea* from Armenia (9b). Compare the stem of the T-marking on the frons, length over which eyes are connected, length of the base of the occipital triangle, and the ante-humeral stripes. Photos: Dietmar Ikemeyer (9a) and Vasil Ananian (9b).

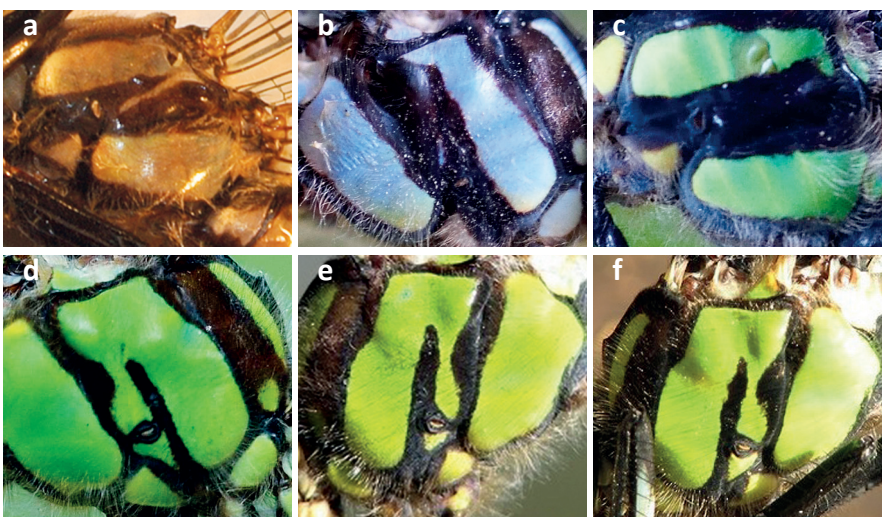

Figure 10. Synthorax of male *Aeshna vercanica* sp. nov. (10a–c) and male *A. cyanea* (10d–f), showing the variation of the colour pattern in the two species. Note the continuous dark band embedding the metastigma in *A. vercanica* sp. nov. from Iran (10b, c), with a small narrow blue-green slit present in the specimen from Azerbaijan (10a), in contrast to an asymmetric black fork left and right of the metastigma in *A. cyanea* specimens from Germany (10d), Tavush province, Armenia (10e) and Lori province, Armenia (10f). Photos: HJD (7a), Dietmar Ikemeyer (10b, c), Dietmar Ikemeyer (10d) and Vasil Ananian (10e, f).

Phenotypical comparison with *A. cyanea*

Aeshna cyanea and *A. vercanica* sp. nov. males show some morphological similarities, in particular the eagle-head shaped upper appendages and the male sexual organs (vesica, hamuli, and lamina). Furthermore, the deeply cleft, sickle-shaped lamina is indistinguishable in the two. However, there are clear morphological and colour differences between the two species. In males the head morphology in particular is different and in females also the appendices and the ovipositor exhibit morphological differences (Tab. 3). Moreover, there are also statistical differences in measurements of wing length and width as well as in pterostigma length and number of cells in the hind wing anal loop and discoidal triangle (Fig. 5).

Notes on biology

Males of *Aeshna vercanica* sp. nov. were observed hunting in the dawn in groups of two to eight individuals, among tree crowns, above bushes, and along forest trails. The first male was seen on a forest trail in the evening and on 22-vi-2013 more individuals were observed in the dawn at the rim of the coastal forest (-10 m a.s.l.). One year later, in July 2014, the species was seen again, at the same sites, hunting in the evening and remaining on the wing despite slight rain. This time also a female was recorded and captured. Other dragonflies in this forest were rare and included just *Caliaeschna microstigma* (Schneider, 1845) and *Cordulegaster vanbrinkae* Lohmann, 1993.

Figure 11. Last abdominal segments and appendages of alive males of *A. vercanica* sp. nov. (11a) and *A. cyanea* (11b) in lateral view. Note in S8–10 the small dorsal spines at the hind margins of each tergite of *A. vercanica* sp. nov., which are absent in *A. cyanea*. Photos: Dietmar Ikemeyer

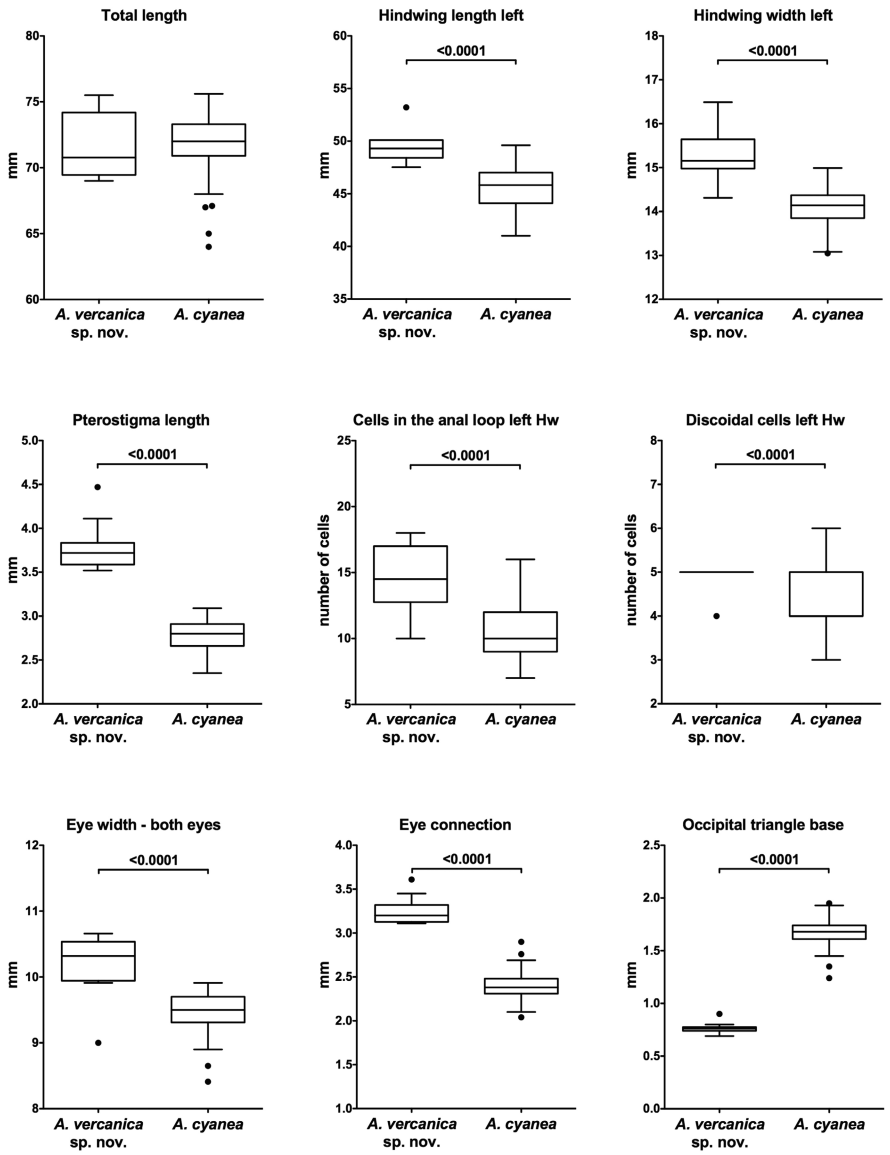

Figure 12. Tukey boxplots (indicating median, first and third quartile, and outliers) of differential morphometric characters between *Aeshna vercanica* sp. nov. males (n = 14) and *A. cyanea* (n = 103), all specimens acetone fixed.

We searched for breeding habitats by searching man-made or natural ponds near and in the forest without success. However, in July 2014 at a spring brook above some small waterfalls in the eastern Golestān province (Fig. 15), approximately 400 km east of the type locality, at about 600 m a.s.l., we found males patrolling short stretches of the brook and flying up rock walls covered with moss. There where often overlapping territories of

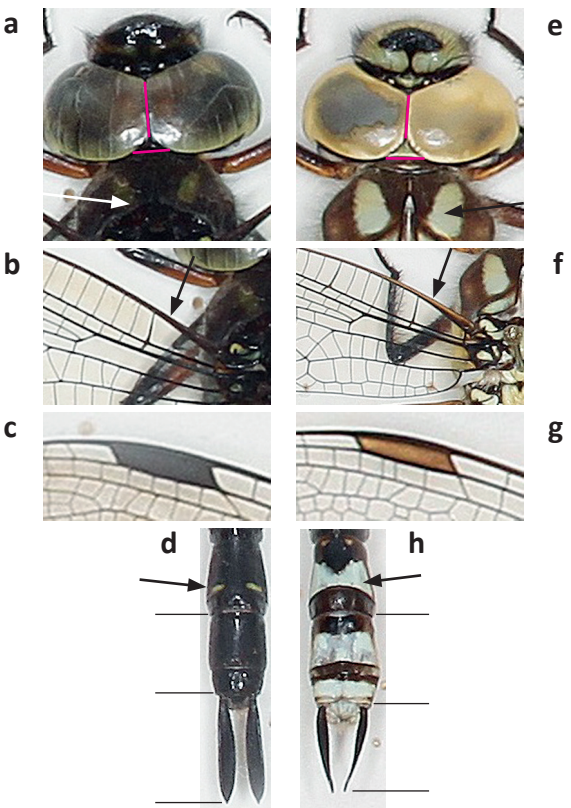

Figure 13. Differential characteristics of the two known females of *Aeshna vercanica* sp. nov. (13a–d) in comparison to *A. cyanea* (13e–h). Shown are head with the T-shaped marking on the frons, length over which eyes are connected, length of the base of the occipital triangle, the ante-humeral stripes (13a, e), the different colour of wing venation (10b, f) and pterostigma (13c, g), the different colour pattern of the last abdominal segments, and the different length of cerci in the two species (13d, h). Photos: Ekkehard Wachmann.

males, in which they did not exhibit agonistic behaviour. A female was observed laying eggs in the moss on the rocks.

Distribution

Caspian Hyrcanian mixed forest in the Golestān and Māzandarān provinces, Iran, and adjacent Talysh Hills in southern Azerbaijan (Fig. 16).

Discussion

Heidari & Dumont (2002) listed four species of *Aeshna* among the 95 dragonfly species recorded from Iran at the time. Recently, a fifth species, *Aeshna serrata* Hagen, 1856, was reported from West Azerbaijan prov-

Figure 14. Structure of the ovipositor of *Aeshna vercanica* sp. nov. (14a) and *A. cyanea* (14b). Indicated are the differences in hind border of basal plate, in the two plates of valvulae 3. Photos: Ekkehard Wachmann.

ince of Iran (Rastegar et al. 2013). *Aeshna cyanea* is known from Europe, the Black Sea fringe of Turkey (Miroğlu et al. 2011), and Armenia (Akramowski 1948; Tailly et al. 2004; Ananian & Tailly 2013). Small, isolated populations are also known from Tunisia (Korbaa et al. 2014) and Algeria (Samraoui & Corbet 2000). A single male was collected by Bodo von Bodemeyer in April 1914 near the Sefid Rud (White River) in Gilān province near Rusht (Ris 1930) and is still the only reported record from Iran. This specimen, kept in the Senckenberg Museum in Frankfurt, Germany, was kindly sent to us by Wolfgang Schneider for examination. It had turned completely black and it could not be ascertained whether it had a T-mark on the frons and a broad single stripe over the synthorax, or a fork as in *A. cyanea*. This character may be absent in this specimen as in *A. vercanica* sp. nov. or may have altered over time during storage. The left hind wing length was 47.9 mm, the pterostigma length 3.5 mm, and the number of discoidal cells in the hind wing was 4. These features are therefore at the

Figure 15. Hyrcanian forest stream where *Aeshna vercanica* sp. nov. males were patrolling and a female was laying eggs. Eastern Golestān province, Iran (20-vii--2014), Photo: Dietmar Ikemeyer

lower limit of *A. vercanica* sp. nov. The specimen was claimed to have been collected in April, which seems very unlikely given the known phenology of *A. cyanea*. In conclusion, we can not determine this specimen with certainty.

A male collected in 2002 in Azerbaijan and described as a blue mutant of *A. cyanea* (Dumont 2004) was, however, found to belong to the new species. Discrimination between *A. vercanica* sp. nov. and *A. cyanea* was further evaluated using 103 males from different parts of the range. Many characters of *A. cyanea* males are stable throughout, as for example the asymmetric fork to the left and right of the metastigma, which is also present in all animals from Armenia. This character may be less pronounced in some individuals but is always clearly recgonizable. The thinner stem of the T-marking was also stable in all *A. cyanea* specimens, with a complete loss of

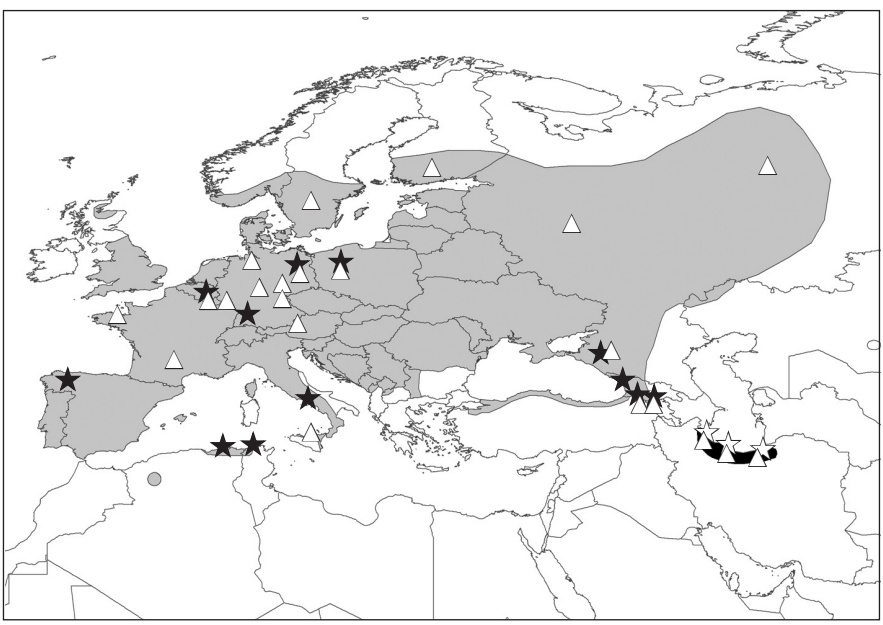

Figure 16. Distribution map of *Aeshna cyanea* (grey; modified after Dijkstra 2006) and the assumed range of *A. vercanica* sp. nov. (black). Triangles indicate localities from where specimens were used for phenotypic characterisation and stars indicate localities from where specimens were used for molecular characterisation.

the stem in some North African specimens. In most males of *A. vercanica* sp. nov., the T-mark was not visible. In the two animals where it could be recognized, the stem was much thicker and shorter even than in extreme variants of *A. cyanea*. In the statistical analysis there was no overlap in the basal length of the occipital triangle and the pterostigma length between the two species. These measurements are of interest as all animals were acetone fixed, which causes a shrinking of 2–3 %, but preserves the colours. Thus, these measurements and colour patterns are comparable. Besides their different colouration, the females of *A. vercanica* sp. nov. are less robustly built than those of *A. cyanea*. Moreover, the terminalia of the females differ, those of *A. cyanea* being shorter than those of *A. vercanica* sp. nov. The hind margin of the ovipositor plate is deeply incised in *A. vercanica* sp. nov., but not in *A. cyanea*.

The base sequence of the COI and ITS revealed genetic distances well above the conventional threshold (ca 3 %) separating species (Hebert et al. 2004). Nevertheless, *A. vercanica* sp. nov. is the closest extant relative to *A. cyanea* and both species may be considered as sisters, sharing a common ancestor. *Aeshna vercanica* sp. nov. is genetically well separated from *A. cyanea* specimens, even more so from the geographically nearest populations in Armenia than from those in the Maghreb. The haplotype anaylsis shows that, under standard settings, the TCS program could not directly link *vercanica* sp. nov. to *cyanea*, suggesting long isolation. The origin of *vercanica* sp. nov. may thus well be pre-Pleistocene. The phylogenetic tree derived from the COI-analysis also shows that *A. viridis* is not a close relative to *A. cyanea*, but is rather allied to *A. grandis*. Further, *A. subarctica* and *A. juncea*, another pair of closely related *Aeshna* species, turn out to be even more closely related than *A. cyanea* and *A. vercanica* sp. nov.

Probably *Aeshna vercanica* sp. nov. occupied the Hyrcanian forest across several ice ages, not expanding from there during interglacials, while *A. cyanea* was pushed back to the Balkans-Caucasus and Maghreb-Iberia at each pleniglacial, but promptly expanded into the ice free zone during each interglacial. Why the former then probably remained sequestered in its Hyrcanian forest throughout the Pleistocene, in contrast to its sister, is of course unknown.

The COI analysis of *A. cyanea* specimens from different localities suggests that animals from the Caucasus (Russian Caucasus, Armenia and Georgia) to southern Russia form a loosely related group, while those in central and southern Europe, with relicts in North Africa, form a subclade linked to the western Mediterranean refuge. This is a familiar situation for postglacially expanding taxa (Hewitt 2000). The Hyrcanian forest is regarded as the cradle of the European forest (Knapp 2005) and the common ancestor of the two species may have evolved in a wooded environment. This could explain why *A. cyanea* is one of the few European dragonflies that can live in dense forest.

The two species also seem to differ in habitat selection. *Aeshna vercanica* sp. nov. appears to be rheophilic, obviously preferring brooks for reproduction, and hitherto was not observed at lentic water bodies at all. On the other hand, the eurytopic *A. cyanea* prefers ponds or other lentic waters for breeding in most of its range, although it also can reproduce in brooks. *Aeshna vercanica* sp. nov. males behave very similarly to males of *Caliaeschna microstigma*, often flying erratically, as well as flying high along torrents rather than slowly along the shore. During hunting and patrolling, *A. vercanica* sp. nov. seems to be gregarious while *A. cyanea* males are territorial (Kaiser 1974).

The range of *A. vercanica* sp. nov. appears to be restricted to the southern margin of the Caspian Sea and the Hyrcanian forest. Its western and eastern limits are somewhat uncertain. However, this region largely overlaps with that of *Cordulegaster vanbrinkae* (Schneider et al. 2013). In fact, we found both species at the same brooks, although *C. vanbrinkae* generally preferred elevations above 700 m a.s.l. while *A. vercanica* sp. nov. remained below 700 m a.s.l.. Between the westernmost locality in the Talysh Mountains and the easternmost locality in the Golestān province there is a distance of about 700 km. The closest *A. cyanea* populations in Armenia are separated from the sites with *A. vercanica* sp. nov. by parts of the Caucasus, the Talysh Mountains, and the northern spur of the Zagros Mountains (Fig. 14). Possibly, there is a gap of about 300-400 km between the two species' ranges.

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