

***Aeshna soneharai* Asahina, 1988, *stat. rev.*,
bona species – an overlooked member
of the European fauna?
(Odonata: Aeshnidae)**

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Abstract. Specimens and observations of *Aeshna mixta* Latreille, 1805, obtained in 2021 from Moscow and Moscow Province, Russia, lead us to the conclusion that what used to be regarded as this well-known Palearctic species in fact represented two species. They differ in details of the abdominal maculation, including the conspicuous dorsal mark on the second segment, the relative length of the male epiproct, and some other characters. In addition, they also differ in the mitochondrial *COI* and *COII* gene sequences (with one odd specimen of *A. mixta* from Balkan Peninsula), but not in the *ITS2* sequence. A potential hybrid male was observed. Analysis of photographic observations on the website “iNaturalist.org” suggests that the true *A. mixta* ranges in North Africa, Europe, the Caucasus, and West Asia, and extends north-east to South Ural and south-eastern Kazakhstan and east to Kashmir. The name available for the second species is *Aeshna soneharai* Asahina, 1988 *stat. rev.*, *bona species*, described from Japan in subspecies rank. This species ranges in East Europe west to the longitude of Moscow and Voronezh, in Ural, Kazakhstan, Siberia, West China, Mongolia, the Far East including Russia, Northeast China, Korea, and Japan. Both species co-occur in Russia between the Don River and South Ural, in Kyrgyzstan and in south-eastern Kazakhstan. The iNaturalist photographs suggest that outside their contact zone, both species (especially

A. mixta in southern Europe) exhibit some variation with respect to almost all characters that are diagnostic in Moscow Province but, on the other hand, are still identifiable using most of these characters. *Aeshna soneharai* seems not to share the swarming behaviour and the migratory abilities of *A. mixta*. The enigmatic *Aeshna lucia* Needham, 1930, is reconsidered a doubtful species rather than a synonym of *A. mixta*.

Further key words: Anisoptera, dragonfly, sympatry, Palaearctic, Russia, Japan, Far East, Korea

Introduction

Aeshna mixta Latreille, 1805, is one of the best studied and most widespread dragonfly species, presumed to range longitudinally from the Atlantic to the Pacific and latitudinally from southern Scandinavia to Northern Maghreb, Levant, Iran Pakistan, Korea, and Japan (KALKMAN *et al.* 2015; DIJKSTRA *et al.* 2020). It is well known for its migratory abilities, including swarm migration to the mountains for aestivation during its pre-reproductive period (SAMRAOUI *et al.* 1998; KALKMAN *et al.* 2015). Based on morphological and structural features, *A. mixta* and the related *Aeshna affinis* Vander Linden, 1820, form a separate clade on phylogenetic trees (VON ELLENRIEDER 2003), deserving isolation into a separate genus yet to be proposed (DIJKSTRA & KALKMAN 2015). *Aeshna lucia* Needham, 1930, so far known only by the male holotype from Beijing, was tentatively supposed to be a synonym of *A. mixta* (ASAHINA 1988), although the detailed original description (NEEDHAM 1930) outlines a quaint combination of characters, with the appendages depicted as in *A. mixta*, the synthorax coloration as in *A. affinis*, a unique coloration of the abdomen, and a black face.

Aeshna affinis and *A. mixta* differ clearly in the male cerci, with a basiventral tubercle in lateral view in the latter. In addition, the synthorax coloration in lateral view is brown with pale stripes in the former *versus* green or bluish-green, with sutures narrowly stressed with black, in the latter (SKVORTSOV 2010; DIJKSTRA *et al.* 2020). Further distinguishing characters usually mentioned are as follows: pterostigmata no longer than 3.5 mm in the former and 4 mm or more in the latter (both sexes); cerci shorter than S9+10 in the former *versus* longer in the latter (females); spots of the dorsal central pair on S3–7 being narrow streaks in the former but rounded and almost as large as those of the posterior pair in the latter (males) (SKVORTSOV 2010).

It is, however, noteworthy that the latter character would not work in the case of the Far Eastern subspecies *A. mixta soneharai* Asahina, 1988, in

which the spots of the central pair on the abdominal segments are rounded. It was described from Nagano Province of Japan and presumed to range also in North and Northeast China and the southern Far East of Russia, *i.e.*, beyond the range of *A. affinis* (ASAHINA 1988; SUGIMURA *et al.* 2021). Other diagnostic characters mentioned for the eastern subspecies were a narrower frontal T-mark and three rows of cells in the male anal loop.

European guides (*e.g.*, DIJKSTRA *et al.* 2020) indicate one more coloration character useful for identification of photographs of mature males in dorsal view: S2 with a conspicuous mid-dorsal, nail-shaped yellow spot in *A. mixta* while in *A. affinis* this pattern element is as blue as the rest of the basic coloration and broader. This character, however, was not recognised by Russian odonatologists (*e.g.*, BELYSHEV 1973; SKVORTSOV 2010), mostly based in Novosibirsk and Moscow, who were familiar with specimens of *A. mixta* with S2 looking as in *A. affinis*, with a blue stripe in place of the yellow 'nail'; such individuals were hitherto not reported from Western and Central Europe. SCHRÖTER (2010) also reported that males of *A. mixta* from Kyrgyzstan were »more bluish and showed less clearly defined dorsal yellow 'nail'-marking on S2« as compared to European specimens. The sixth author (AS) attracted attention to occurrence of such individuals in *A. mixta* from Russia, so this fact was finally mentioned in two most recent books on the European dragonfly fauna (DIJKSTRA *et al.* 2020; ONISHKO & KOSTERIN 2021).

In 2021, the first author (VVO) encountered both versions of males of 'A. mixta', with yellow and blue S2 central mark, within the city of Moscow. As outlined in this study in detail, they appeared to differ also in several characters concerning morphology, maculation, behaviour, flight period, and mtDNA sequences. We consider differences between those two phenotypes big enough to claim, admittedly with some degree of uncertainty, that they can be regarded as separate species: the south-western *A. mixta* and the eastern *A. soneharai* *stat. rev.*, which are co-occurring in sympatry in the Russian Plain, in South Ural and in south-eastern Kazakhstan. While the arguments for this point of view will be delivered in this study subsequently, to simplify presentation, we will use these two species names in this paper as taken for granted, instead of speaking at first on conventional entities such as 'versions' or 'forms' which would be abandoned at the end of the paper.

Material and methods

VVO is conducting a regular dragonfly monitoring of several habitats in Moscow City and in the Moscow Province of Russia, including observing of behaviour, photographing, and collecting voucher specimens. In the 2021 season, this allowed him to recognise and observe dragonflies of two species considered distinct in this paper – *A. mixta* and *A. soneharai* – in the field, and to sample voucher specimens.

Individuals were photographed in nature with an iPhone 7, all individuals captured by net were photographed alive in hand and most of them were released afterwards, with a few being collected as voucher specimens. For illustrations, mostly photographs of alive individuals in hand were used as they show the coloration details best. Morphological details were photographed with a Zeiss Stemi 2000-C microscope equipped with a Canon PowerShot A640 camera; their illustrations were obtained from serial photos with shifted focus with the program Helicon Focus 5.3.

Geographic distribution of *A. mixta* and *A. soneharai* was assessed by revising numerous photographic observations, identified as *A. mixta*, in the iNATURALIST (2021) platform, which are also adopted by the Global Biodiversity Information Facility (GBIF; iNATURALIST RESEARCH GRADE OBSERVATIONS 2021). The link to an iNaturalist observation has the following format: <http://www.inaturalist.org/observations/x>, where 'x' is its unique numeral (of variable number of digits). These unique numerals are provided in parentheses in the text below where necessary. The schematic map of distribution of both species was composed from coordinates provided in observations using the software NextGIS QGIS version: 20.8.0. The observations which represent *A. soneharai* will be re-identified and the photos serving as the material of this paper will be submitted after the species rank of *A. soneharai* is recognised and adopted as a community name in iNaturalist. The specimens discussed are deposited in the authors' collections.

Abbreviations

COI – mitochondrial cytochrome oxidase subunit I gene;

COII – mitochondrial cytochrome oxidase subunit II gene;

ITS2 – transcribed spacer 2 in the ribosomal RNA cluster (nuclear).

Total DNA was extracted from the femur muscle tissue of seven acetoned and dried specimens using DNeasy Blood and Tissue Kit (QIAGEN, Germany) according to the manufacturer's protocol. The following sets of PCR primer pairs were used to amplify partial nucleotide sequences of *COI* and *COII* mitochondrial genes, as well as the complete nucleotide sequence of the nuclear *ITS2* region:

- for the 858 b.p. long *COI* 5'-fragment (including the barcoding region proposed by FOLMER *et al.* 1994) – original primers worked out to match sequences of Orthoptera: 911.1 (not published):
5' – CAACAAACCATAAGGATATTGG – 3' and
912.4 5' – GTTGCTGATGTAAAGTATGC – 3';
- for the 569 b.p. long *COII* fragment (SIMON *et al.* 1994):
C2J 5' – AGAGCTTCTCCTTTAATAGAACA – 3' and
C2N 5' – CCACAAATTTCTGAACATTGACCA – 3';
- for *ITS2* (PORTER *et al.* 1991; WALTON *et al.* 1999):
2A 5' – TGTGAACTGCAGGACACAT – 3' and
2B 5' – TATGCTTAAATTCAGGGGG – 3'.

PCR amplification was performed in 20 µl volume containing 0.1 µg of genomic DNA, 10 mM Tris HCl (pH 8.9), 1 mM (NH₄)SO₄, 1.5 mM MgCl₂, 200 µM of each of four dNTPs, 0.5 µM primers, and 2.5 units of Tag DNA polymerase. After an initial denaturation step for 2 min at 94°C, the PCR samples were subject to 30 cycles of amplification consisting of 30 s denaturation at 94°C, 30 s annealing at 46°C for *COI* and *ITS2* sequences, 44°C for *COII* sequences, 1 min elongation at 72°C. Final elongation was carried out at 72°C for 10 min. The PCR products were analysed in agarose gel electrophoresis, extracted from gel with a QIAquick gel Extraction Kit (QIAGEN).

The sequences obtained were deposited to GenBank under the following accession numbers: OM089771–OM089777 for the *COI* fragment, OM100713–OM100719 for the *COII* fragment, OM102545–OM102551 for *ITS2*.

The following sequences were adopted from GenBank for phylogenetic analysis: KJ873232, HM422047, MT298232, KF257096, AB708592, MW208416, MW490185, MZ658835, MT298248, MT298246, KY847570, KC912204, MW208419, KF257093, MZ659598 of *COI* and JN615363 and EU055343 of *COII*.

Alignment of sequences obtained experimentally and from GenBank was made using MAFFT v7.312 program (KATOY & STANDLEY 2013) with the following parameters “--localpair--maxiterate 1000”.

Phylogenetic trees were obtained via the Bayesian analysis using MrBayes 3.2.6 program (HUELSENBECK & RONQUIST 2021; RONQUIST & HUELSENBECK 2003). GTR+G model was used in all Bayesian analyses, which was predicted based on Akaike Information Criterion (AKAIKE 1974). For each set of sequences, the starting running parameters were as follows: 1 million generations (ngen), sampling every 100 generations (samplefreq), with eight Markov chains Monte Carlo (nchains=8) and temperature 0.2 (temp). The analysis continued for additional generations until three conditions were satisfied: (i) average standard deviation ≤ 0.01 , (ii) no tendency of increase or decrease over time on the MrBayes 3.2.6 sump plot, and (iii) Potential Scale Reduction Factor (PSRF) values for were close to 1.0. For every set of sequences all PSRF values differed by less than 0.05. In the end of analysis, we discarded 25 % of the trees.

In addition to the Bayesian analysis, we applied the Maximum Likelihood method using the IQ-tree software (TRIFINDOPOULOS *et al.* 2016). GTR+G+I model was used in all Maximum Likelihood analyses, which was predicted based on Akaike Information Criterion (AKAIKE 1974). Two statistical tests available at IQ-tree were applied to evaluate the credibility of the phylogenetic clusters: SH-like aLRT and ultrafast bootstrap (UfBoot).

For each tree a set of three support values were obtained: Bayesian posterior probabilities, SH-like aLRT and UfBoot. We choose the limit of 80 % of the former for branches to be shown in phylogenetic illustrations.

For calculating pairwise distance, MEGAX v.11 software (KUMAR *et al.* 2018) was used, with Tamura-Nei method, gamma distribution (G) set to 1.00, and gaps sites treated as complete deletion.

Records of *Aeshna mixta* and *A. soneharai* in Moscow and Moscow Province in 2021

***Aeshna mixta* Latreille, 1805**

(Figs 1–2 left, 3a, c, f)

1♀ collected and sequenced (Fig. 6a, specimen MSU2, sequences OM089774, OM100716, OM102548), 1♂ photographed in hand (Fig. 1c left), Russia, Moscow, the ponds at the library of Moscow State University, 55.696° N, 37.520° E,

16.viii.2021; 2♂ collected (Figs 1a, b, d, e left, 3a, c), 1 of them sequenced (specimen MSU1, sequences OM089772, OM100714, OM102546), 2♂ sighted, the same place, 30.viii.2021; 1♀ collected (Figs 2 left, 3f, 6b), 1♂ photographed in hand, 3♂ sighted, the same place, 13.ix.2021; 2♂ collected, 1 of them sequenced (specimen MnF1, sequences OM089775, OM100717, OM102549), 2♂ sighted, Russia, Moscow, Mnevnikovskaya Poyma [Mnevniki floodplain], 55.759° N, 37.464° E, 24.viii.2021; 1♂ 1♀ (copula) photographed in hand, 3♂ sighted, Russia, Moscow Province, Uzunovo village, Lake Aerodromnoe, 54.523° N, 38.594° E, 14.viii.2021.

Aeshna soneharai* Asahina, 1988, *stat. rev.

(Figs 1–2 right, 3b, d, e, g, 4)

1♂ photographed in hand, Russia, Moscow, the ponds at the library of Moscow State University, 55.696° N, 37.520° E, 25.vii.2021; 2♂ 2♀ photographed in hand, 2♂ photographed in nature, many ♂ sighted, the same place, 31.vii.2021; 1♂ photographed in hand, many ♂ sighted, the same place, 09.viii.2021; 1♀ collected and sequenced (specimen MSU4, sequences OM089773, OM100715, OM102547), 5♂ (Fig. 1c right), 1♀ photographed in hand, 2♂, 1♀ photographed in nature, many ♂ sighted, the same place, 16.viii.2021; 4♂ 3♀ (Fig. 6b, c) photographed in hand, the same place, 18.viii.2021; 2♂ photographed in hand, copula photographed (Fig. 4), many ♂ sighted, the same place, 25.viii.2021; 1♂ collected (Figs 1, a, b, d, e right, 3b–e) and sequenced (specimen MSU3, sequences OM089771, OM100713, OM102545), 1♂ photographed in hand, 1♂ photographed in nature, many ♂ sighted, the same place, 30.viii.2021; 2♂ 2♀ (Figs 2 right, 3g) photographed in hand, 2♂ photographed in nature, the same place, 13.ix.2021; 1♂ collected and sequenced (specimen MnF2, sequences OM089776, OM100718, OM102550), 3♂ photographed in nature, *ca* 10♂ sighted, Russia, Moscow, Mnevnikovskaya Poyma [Mnevniki Floodplain], 55.759° N, 37.464° E, 24.viii.2021; 5♂ photographed in hand, Russia, Moscow, Lake Chernoe, 55.727° N, 37.845° E, 15.viii.2021; 1♂ photographed in hand, 2♂ sighted, Russia, Moscow Province, near Ozherelki village, 55.849° N, 38.845° E, 12.ix.2021; many ♂ sighted, Russia, Moscow Province, Khorlovo village environs, 55.327° N, 38.795° E, 26.vii.2021; 1♀ photographed in hand, Russia, Moscow Province, Uzunovo village, Lake Aerodromnoe, 54.523° N, 38.594° E, 14.viii.2021.

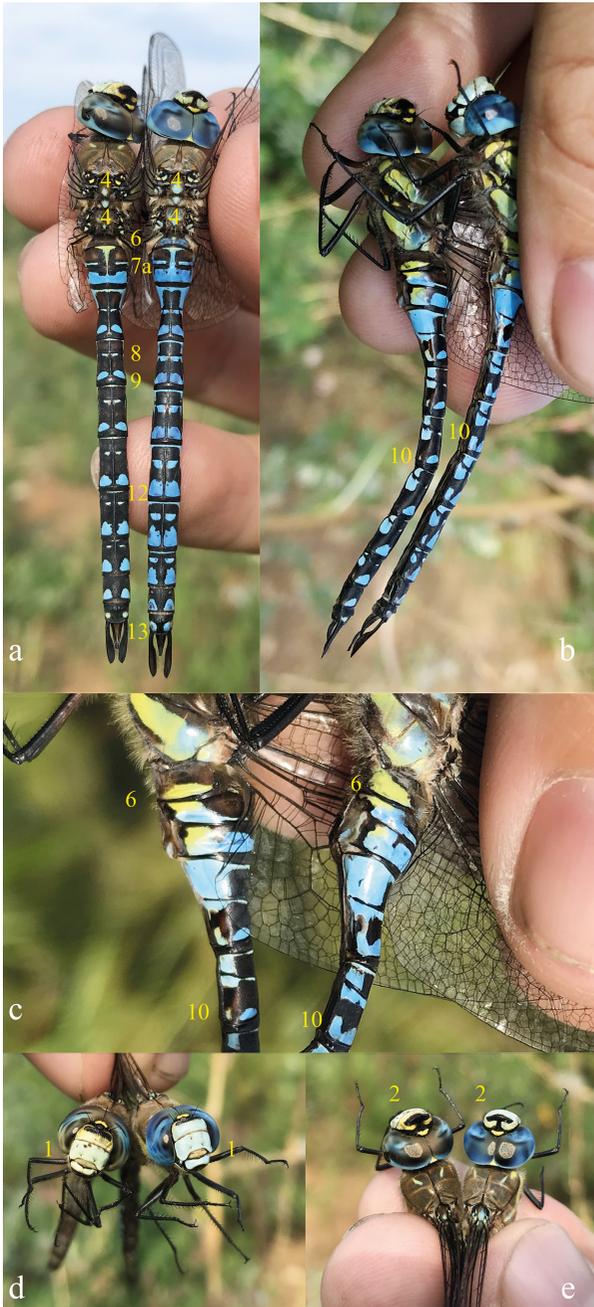


Figure 1. Pairwise comparison of males of *Aeshna mixta* (left in each pair) and *A. soneharai* (right in each pair) simultaneously obtained at the ponds at the library of Moscow State University, a, b, d, e on 30.viii.2021 (the male of *A. soneharai* sequenced, specimen MSU3), c on 16.viii.2021; a – general habitus in dorsal view; b – ditto in lateral view; c – bases of abdomen in lateral view; d – head in frontal view; e – head in dorsal view (mirrored). Diagnostic characters are indicated with their numerals in Table 1. Not to scale.



Figure 2. Pairwise comparison of females of *Aeshna mixta* (left in each pair) and *A. soneharai* (right in each pair) simultaneously obtained at the ponds at the library of Moscow State University on 13.ix.2021; a – general habitus in dorsal view; b – ditto in lateral view; d – head in dorsal view; e – head in frontal view. Diagnostic characters are indicated with their numerals in Table 1. Not to scale.

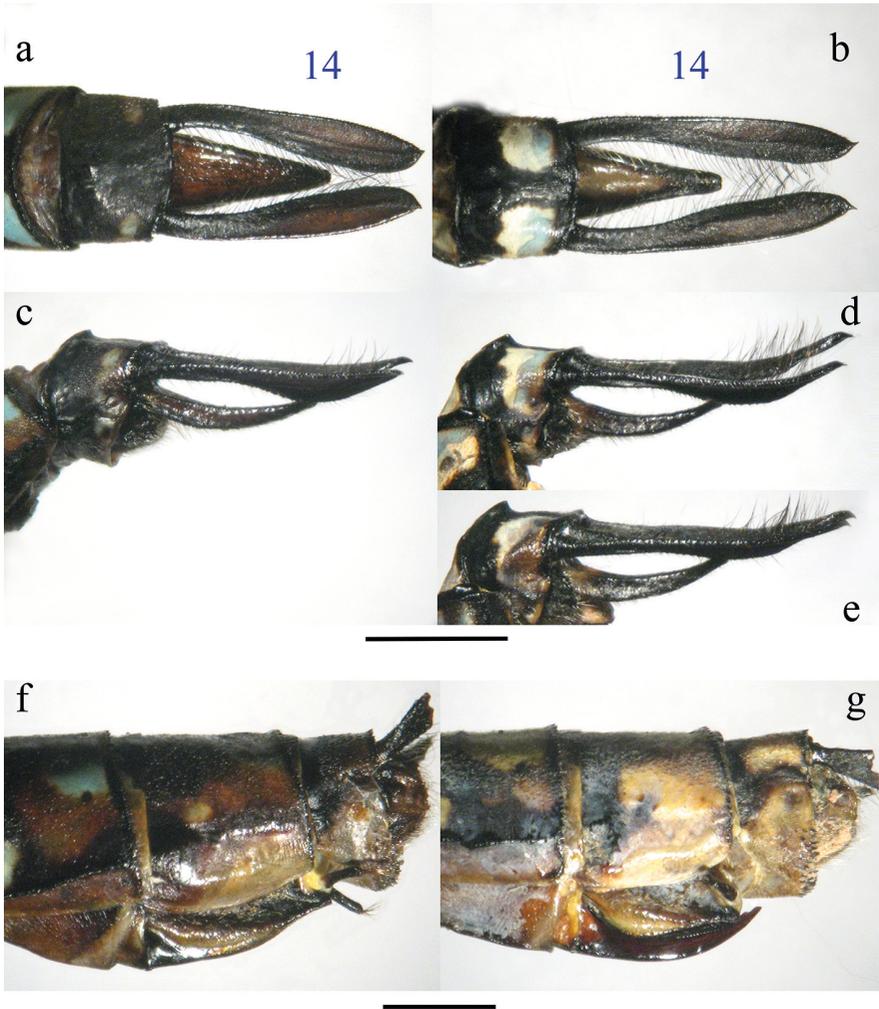


Figure 3. Details of abdominal terminalia of males (a–e) and females (f–g) of *Aeshna mixta* (a, c, f) and *A. soneharai* (b, d–e, g) simultaneously obtained at the ponds at the library of Moscow State University on 30.viii.2021: a, b – appendages in dorsal view; c–e – ditto in lateral view; f, g – end of abdomen in lateral view. The male of *A. mixta* shown in a and c (specimen MSU1) and both females (specimens MSU2 and MSU4 of respectively *A. mixta* and *A. soneharai*) sequenced. 14 stands for the characters in Table 1 (the relative epiproct length). Scale bar 2 mm.

***Aeshna* sp., with combination of characters of *A. mixta* and *A. soneharai*;
see below**

(Figs 5a, b)

1♂ photographed in hand (Figs 5a, b, not collected), Moscow, the ponds at the library of Moscow State University, 55.696° N, 37.520° E, 09.viii.2021

Copula male *A. soneharai* / female *A. mixta*

(Figs 5c–d)

1 copula photographed in hand, the same place and date as above.

Thus, in the 2021 season, VVO found *A. mixta* and *A. soneharai* together in two of three examined sites within the Moscow City limits and in one of three



Figure 4. A copula of *Aeshna soneharai* at the ponds at the library of Moscow State University. Photo: VVO (25.viii.2021). Not to scale.

examined sites in Moscow Province, while in the remaining sites (three of six examined) only *A. soneharai* was found. In general, *A. soneharai* was much more numerous than *A. mixta*, so that among individuals photographed or collected 50 represented the former and only ten represented the latter, plus one male with intermediate characters (see below). At the regularly monitored ponds at the Moscow State University library, *A. soneharai* was observed first on 25 July, while *A. mixta* was observed first on 14 August. Many copulae of *A. soneharai* were sighted (Fig. 4), one consisting of a male of this species and a female of *A. mixta* (Figs 5b, c), but none of *mixta*. In previous 2015–2020 years, VVO observed in Moscow and Moscow Province only *A. soneharai*, with 13 observations in total (that means less active observing those years).

Differences between *Aeshna mixta* and *A. soneharai* in morphology and coloration in the Moscow area

The differences in morphology and maculation between two groups of individuals from the Moscow area are enumerated in Table 1, with the most conspicuous and easily scored ones boldfaced, and illustrated in Figures 1–3. Two distinct complexes of several distinguishing characters of both sexes, some of which are conspicuous, were alternatively exhibited by all specimens examined and 48 out of 49 individuals photographed in 2021 in Moscow and Moscow Province. Both character complexes were simultaneously found in the same localities. We find this a convincing argument to consider that two sympatric, distinct species were involved. We should stress that Table 1 concerns only differences observed in specimens collected in sympatry in Moscow and Moscow Province, where they are steady except for variable characters 3 (inner cells in the anal loop) and 13 (general size), which are variable but included since mentioned by ASAHINA (1988). Validity of the characters of Table 1 elsewhere will be discussed below. However, Table 1 comes along with three comments with respect to the original description of *A. mixta soneharai* from Japan by ASAHINA (1988).

The species with a ‘nail’ on S2 (yellow in males) and narrow transversal central spots on S3–8 (Figs 1, 2 left) fits well *Aeshna mixta s. str.*, as defined in European guides (e.g., DIJKSTRA *et al.* 2020).

Four of the characters enumerated in Table 1 for the species with a stripe on S2 (blue in males) and broad central spots on S3–8 (Figs 1, 2 right) were

mentioned – although with somewhat different formulations (see comments to Table 1) as diagnostic in the original description of *A. mixta soneharai* (ASAHINA 1988). These are the characters no 2, 3, 8 and 14 in Table 1, namely: the narrower T-like postfrontal spot; appearance of inner cells in



Figure 5. A presumed male hybrid of *Aeshna soneharai* and *A. mixta* (a, b) and a copula of a male of *A. soneharai* and a female of *A. mixta* (c, d) at the ponds at the library of Moscow State University. Photos: VVO (09.viii.2021). Not to scale.



Figure 6. Live coloration of females of *Aeshna mixta* (a, b) and *A. soneharai* (c, d) photographed at the ponds at the library of Moscow State University on 16.viii.2021 (a; sequenced, specimen MSU2), 18.vii.2021 (b, c), 13.ix.2021 (d). Diagnostic characters are indicated with their numerals in Table 1. Lateral view; not to scale.

the anal loop; broad, more or less rounded spots of the central dorsal pair on most of abdominal segments; and longer male cerci. Other characters from Table 1 were not compared by ASAHINA (1988). The book by SUGIMURA *et al.* (2021) and an internet site by AOKI (2021) exhibits photographs of males, in lateral and dorsal views, and a female (lateral view) of *soneharai* from Japan. They correspond to the Moscow specimens in important characters such as the S1 lateral pattern (character 6), the S2 dorsal pattern (character 7), the large posterior dorsal spots on S3–8 in male (character 9) and postero-ventral spots on the S3–6 tergites in male (character 10). Spots of the central dorsal pair on S3–8 (character 8) in the Japanese male are very small (as in *mixta s. str.*, vs large in Moscow specimens) but characteristically roundish as in Moscow specimens, rather than very narrow in *mixta s. str.* The same is shown in the original description of *soneharai* (ASAHINA 1988: figs 15, 18, 21). So ‘the second Moscow species’ corresponds to the original description of *soneharai* in all respects, but differs from it as well as from *mixta s. str.* in much larger size of the central dorsal spots on abdominal segments, which, however, are rounded as in *soneharai*. Despite this, we find it possible to identify this species as *soneharai* and therefore we preliminarily rise the rank of this taxon to species level:

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The original description of *soneharai* by ASAHINA (1988: 68) contains one more diagnostic character described *verbatim* as follows: »Female basivalvula of ovipositor longer, lateral vulva shorter, the latter not extending over 9th segment«. At the same time, the relevant figure 30 therein does not show this character, so that the drawings of the female ovipositor of *soneharai* and a European *A. mixta* do not show any difference. The Manchurian females attributed to *soneharai* were said to have »female basivalvula short, lateral vulvae short«. Unfortunately, we collected in Moscow only one female specimen each of *A. mixta* and *A. soneharai*, which, however, strikingly exhibited the same difference as mentioned by ASAHINA (1988: 68): the genital valvulae V1 (according to PFAU 1991) strongly protruded beyond the genital valvulae V3 and the S9 posterior margin in *A. soneharai* (Fig. 3g), while is not at all protruding in *A. mixta* (Fig. 3f). However, this appeared to be an artefact of a movable organ position, the correspondence between the statement

by ASAHINA (1988) and our specimens being a matter of chance – this could also explain the controversy between text and figure in ASAHINA (1988). Examination of a series of females corresponding to both species but originating from Kazakhstan and West Siberia (results not shown), in the collection of the Institute of Systematics and Ecology of Animals of the Siberian Division of the Russian Academy of Sciences, Novosibirsk, showed that the genital valvulae V1 are normally appressed to the abdomen ('hidden') and in this position their tips do not extend beyond the genital valvulae V3 and the S9 hind margins. They also could be raised to a different degree, either completely taken out from the scabbard of the genital V3 and seen for their full length, or still partly hidden behind V3 but with their tips protruding behind V3 as in Figure 3g.

Among 49 individuals collected or photographed in Moscow and Moscow Province, only one male photographed on 09.viii.2021 had a complex of characters which did not fit either *A. mixta* or *A. soneharai* (Figs 5a, b). It had S1 sides and a blue dorsal stripe on S2 as in *A. soneharai*, but the latter was, however, unusually broadened posteriorly. Additionally, the central dorsal spots on abdominal segments were intermediate between the species in size, shape (broadly triangular), and colour (bluish); and the posterior lateral spots of the abdominal segments were somewhat smaller than in 'typical' *A. soneharai*. This male could be interpreted as an F₁ hybrid between the two species. Curiously, on the same date and in the same place, a copula was photographed (Figs 5c, d) composed of a male of *A. soneharai* and a female of *A. mixta* (see the very characteristic difference in the S1 lateral coloration, character 6), which shows how such hybrids may possibly originate.

In 2021, the coloration of alive individuals was photographically registered in total in four females of *A. mixta* and 25 females of *A. soneharai* from Moscow and Moscow Province. The females of *A. soneharai* were distinctly dimorphic (Figs 6c, d). The majority of them had the pale elements of abdominal maculation greenish-yellow to yellow (Fig. 6c), while two of three females photographed on 18.viii.2021 (8% of the total) were androchromatic, having these elements bright-blue, as in males (only sides of S1 and S2 have some yellow shades; Fig. 6d). All the four females of *A. mixta* looked

Table 1. The differences between Moscow specimens of *Aeshna mixta* and *A. soneharai* in morphology and maculation. The most conspicuous traits are boldfaced and indicated in Figures 1–3 and 6. * – traits mentioned in the original diagnosis of *A. mixta soneharai* (ASAHINA 1988); ! – a trait partly not exhibited by its type series.

| No. sex | body part | <i>A. mixta</i> | <i>A. soneharai</i> |
|----------------------|---|--|---|
| 1 male (ma- ture) | face frontal surface (Fig. 1d) | yellowish | whitish to bluish |
| 2 both | T-like mark on post-frons (Figs 1e, 2c) | stout, with a stout 'stem' | * narrower, with a slender 'stem' |
| 3 both | anal loop | without inner cells | * one or (rarely) two inner cells often present, but may be absent (see comment 1) |
| 4 both | costal plate (an inter-alar sclerite at the costal vein base) of both wings (Figs 1a, 2a) | with an entire pale spot, more conspicuous on fore wing | with the pale spot split into pieces, distinctly on fore wing, less distinctly on hind wing |
| 5 male | S1 dorsal side (Fig. 1a) | with no or obscure yellowish markings | with three distinct blue spots which may fuse into a complete ring |
| 6 both | lateral pale spots of S1 adjacent to posterior margin of this segment (Figs 1c, 2b, 6) | tapering beneath, usually of approximately semicircular (lunular) shape | strongly expanding beneath to occupy the lower tergite margin, usually as a right-angled triangle, adjacent to the tergite posterior and ventral margins |
| 7a male | dorsal pale maculation of S2 (Fig. 1a) | a broad, yellow, nail-shaped mid-dorsal spot at S2 anterior margin, with a pointed central caudad projection, either not connected to blue ring at posterior margin, or connected to it with a narrow pale yellowish line | a broad blue mid-dorsal stripe, not or barely tapering posteriorly and connecting to blue ring at posterior margin |
| 7b female | dorsal pale maculation of S2 (Fig. 2a) | a pointed triangular, nail-shaped yellow mid-dorsal spot at S2 anterior margin, continued to posterior margin as a line, often interrupted | a yellow mid-dorsal stripe, bordered with black, of even width expect for being strongly expanded at anterior margin and pointed at posterior margin |

| No. sex | body part | <i>A. mixta</i> | <i>A. soneharai</i> |
|-------------------------|--|---|--|
| 8 both | central pair of dorsal pale spots on S3–8 (Figs 1a, 2a, 6) | small and very narrow, as triangular or oval transversal strokes, yellowish in males | large and broad, trapezoid to semicircular, blue in males (see comment 2) |
| 9 both | posterior pair of pale dorsal spots on S3–8 (Figs 1a, 2a) | smaller, their length changes in posterior direction from <i>ca</i> 12–14 to 35–37 % of tergite length; separated from each other with a broader gap. | larger, their length changes from <i>ca</i> 17–50 % of tergite length; set very closely to each other, |
| 10 both | postero-ventral pair of pale spots on tergites of S3–6 (Figs 1b, c, 2b, 6) | small to vestigial or totally missing at S5–6, in males yellowish | large, on S3–4 almost as large as anterior spots, in males blue |
| 11 male | posterior tergite margins at S3–S8 (Fig. 1a) | with yellow streaks interrupted at sides, shorter in posterior segments | with complete blue rings |
| 12 male | dorsal pair pale spots on S10 (Fig. 1a) | from small, yellowish, or dull whitish to completely missing | large, blue |
| 13 male (♀ not studied) | cerci (measured in dorsal view, from the centre of S10 hind margin) | slightly shorter; <i>ca</i> 2.08 the length of S10 | *slightly longer; <i>ca</i> 2.25 the length of S10 (see comment 3) |
| 14 male | epiproct | relatively longer; <i>ca</i> 62–65 % of cercus length, <i>ca</i> 1.50 the length of S10 (Fig. 3a) | relatively shorter; <i>ca</i> 45–52 % of cercus length, <i>ca</i> 1.25 the length of S10 (Fig. 3b) |

Comments to Table 1:

1. This character of *soneharai* was described by ASAHINA (1988: 68) as »three cell rows in males and two cell rows in females«. In Moscow, we did find inner cells variably present (or absent) in the anal loop in *A. soneharai*, in both sexes, but did not see individuals of *A. mixta* having them.
2. In the type series of *soneharai* (ASAHINA 1988) and other specimens of Japan these spots are broad and roundish but small, so corresponding to the Moscow specimens in shape but not in size.
3. ASAHINA (1988) provided absolute values (problematic as correlating with the general size) for the cercus length: 5.0–5.5 mm in Japanese males, 4.8–5.5 mm in continental males, 6.0–6.5 mm in Japanese females, 5.0–5.5 in continental females. This character 13 is weak and seems to be unreliable but is included as considered by ASAHINA (1988).

‘intermediate’ in coloration, with yellow and blue shades variably present in the pale abdominal macula (Fig. 6a, b). Of course, not enough females of *A. mixta* were examined to rule out occurrence of androchromatic ones in this species in Moscow.

Molecular comparison of *Aeshna mixta* and *A. soneharai*

Three specimens (2♂ 1♀) identified as *A. mixta* from Moscow, and the same number of specimens of each sex from the same places identified as *A. soneharai*, were taken for molecular analysis. Also, we included an immature male specimen from Iran (Ostan-e Lorestan, Shakhrestan-e Khorramabad, a gorge 2.2 km WNW of Pasil village, 33.35043544° N, 48.85138571° E, 1 460–1 500 m a.s.l., 26.v.2017; sequences OM089777, OM100719, OM102551) (KOSTERIN & AHMADI 2018) to confirm its identification as *A. mixta* by the epiproct relative length, as it was too immature to be reliably identified by the colour pattern.

Phylogenetic trees were reconstructed with both Bayesian and Maximum Likelihood methods for the sequences obtained for the *COI* fragment, as well as ten sequences of the same fragment available in Gene Bank for *A. mixta*, including two identified as *A. mixta soneharai* from Japan (see KARUBE *et al.* 2012), and three sequences of the most closely related *A. affinis*; *Aeshna crenata* Hagen, 1856, and *A. subarctica* Walker, 1908, were used for the out-group. Figure 7 shows the Bayesian tree (as well as the node support for the ML tree). The sequences of *A. soneharai* were nearly identical: three sequences from Moscow differed from two sequences from Japan with just one substitution, which is striking considering the geographic distance. As a result, they formed a well-supported cluster. The sequence alignment (not shown) revealed them to share nine ‘diagnostic’ nucleotide substitutions not found elsewhere in other sequences involved. They comprised 1 % of 858 nucleotide positions of the fragment sequenced. The cluster of ‘the true’ *A. mixta* is not so well supported (Fig. 7), since the sequence MW208416 from a specimen from Bosnia and Herzegovina appears as a branch of its own (actually it clusters with *A. mixta* with a low posterior probability of 70 %, so this node is not shown in Fig. 7). This is because this sequence, as well as those from Sardinia and Finland, variably share few nucleotide substitutions with *A. soneharai* (not counted above among the ‘diagnostic’ ones

for the latter). Among sequences of *A. mixta*, the greatest pairwise genetic distance of 0.75 % was between Germany_KY847570 and Bosnia_Herzegovina_MW208416 and the same between Germany_KY847570 and Sardinia_MW490185, while the distance between the Moscow and Japanese *A. soneharai* was 0.02 %. The pairwise distances between any sequence of *A. soneharai* and different sequences of *A. mixta* were smallest, 1.79 %, in the case of the enigmatic sequence Bosnia_Herzegovina_MW208416, and the second small, 2.06 %, in the case of Sardinia_MW490185, while the greatest distance, 2.88 % was in the case of Germany_KY847570. So, the minimal distance between species (even considering the out-of-cluster *mixta* from Bosnia and Herzegovina) was 2.4 times greater than the maximum intraspecies distance. With our small sample we cannot deduce more information from distances.

The sequenced *COII* fragment (489 b.p. were sequenced of the 589 b.p.-long amplified fragment) has no matches from *A. mixta* and *A. affinis* in Gene Bank, so the tree of Figure 8 includes only our sequences and the outgroup of Anisoptera sp. and *Rhionaeschna multicolor* Hagen, 1861, from Gene Bank. In this tree, both clusters, of *A. soneharai* and *A. mixta*, are very well supported. This is because the sequenced 489 b.p. long fragment differed between these species in as many as 11 substitutions (2.58 % genetic distance) and was identical within each species (not shown). However, in case of *COII* our current sampling is truly poor.

In both reconstructions, the specimen from Iran, identified as *A. mixta* by the appendage structure, indeed grouped with *A. mixta*.

The sequences of *ITS2* obtained from *A. mixta* and *A. soneharai*, as well as the sequence AB711411 from Gene Bank of *A. mixta*, appeared to be identical (no sequences of *A. affinis* are so far available).

Geographic distribution of *Aeshna mixta* and *A. soneharai*

Specimens with the combination of most important characters of *A. soneharai* have never been reported from West and Central Europe, which appear to be inhabited with *A. mixta* only. On the other hand, all specimens so far available to us from Ural, Siberia, and the Far East of Russia appeared to be *A. soneharai*, hitherto mistaken for *A. mixta*. So, all Russian literature mentioning *A. mixta* for the Asian parts of Russia in fact referred to

A. soneharai. It appeared necessary to find both species in the same localities, which happened in 2021 in the capital of Russia, to get an insight that two species seem to have been lumped under *A. mixta*. The question arises of the geographic distribution of these two, first in the European part of Russia, and Middle and Central Asia. Since most of their external diagnostic



Figure 7. Phylogenetic tree of *Aeshna* spp. reconstructed by the Bayesian method from partial nucleotide sequences of the *COI* mitochondrial gene. The node support values are shown as follows: Bayesian posterior probability (in percent, for uniformity) / aLRT support / UF-bootstrap support. Black circles indicate sequences obtained in the current work; sequences without circles are obtained from Gene Bank, with respective accession numbers indicated.

characters are conspicuous (Table 1) and easy to identify on photographs, we utilised an excellent opportunity to infer their geographical distribution from the photographic data contained in iNATURALIST (2021), a popular citizen science platform, which for the time of our analysis contained 3 016 observations identified as *A. mixta*. The result is presented on a map of Figure 9, which also includes points where identification by photographs was uncertain.

From these data (Fig. 9), *A. mixta* appeared to range in North Africa, Europe, the Caucasus, and Western Asia from which its range extends to Kashmir – from where *mixta s. str.* was reported still by ASAHINA (1988). In the Russian Plain it occupies the north-western Leningrad and Pskov Provinces; south of them it extends to the east to the Volga River between Kazan' and Samara and reaches Voronezh Province in the south-east. Besides, there is an observation of a female 52167497 from Bashkortostan, Iglino village (54.93° N, 56.45° E), which is actually in southern Ural, and therefore the easternmost observation of *A. mixta* in Russia. There is no record for either of the two species in the southern part of the Don and Volga interfluvium between Voronezh Province and the Caucasus. There are just two records of *A. mixta* in Kazakhstan, both in its south-eastern part – at Lake Alakol (observations 57902967 and 57902969 by Ruslan Nurkhanov) and in the Tysh-



Figure 8. Phylogenetic tree of *Aeshna* spp. reconstructed by the Bayesian method from partial nucleotide sequences of the *COII* mitochondrial gene. Designations as in Figure 7.

kantau Massif Southern Dzhungarian Alatau Mts (observation 101009833 of a female by OEK).

According to iNaturalist observations (Fig. 9), *A. soneharai* occupies Korea, Northeast and Northwest China, Mongolia, foothills of the Pamir-Alai and Tian Shan Mts including Dzungarian Alatau, the Asian part of Russia north to Khabarovsk, Irkutsk, Krasnoyarsk, Tomsk, Omsk and Toblosk, South Ural, and the European part of Russia west to Moscow and Voronezh (observation 65743135) Provinces, and south to Volgograd (observations 36496931 and 59426943). Although it occurs there, no observations of *A. soneharai* from Japan are lodged in iNaturalist.

So, the ranges of both species overlap in Russia in the territory between Moscow Province in the north-west (the data presented above), Voronezh Province in the south-west (doubtless observations of females 100075324 of *A. mixta* and 65743135 of *A. soneharai* from the same Kalach District, both by Konstantin Hatkovsky), Tatarstan in the east (a female observation 102461056 of *A. mixta* from Laishevo District and seven observations of *A. soneharai*), and most probably also in the South Ural from where only one record of *A. mixta* exists from Bashkortostan (see above). Another area of co-occurrence is in south-eastern Kazakhstan. The situation in the eastern half of Iran and Central Asia including Xinjiang is unclear because of scarcity of data. The males from the Alamedin Canyon in Kyrgyz Alatau illustrated by SCHRÖTER (2010: fig. 22) were *A. soneharai* (characters 4, 6, 8), while two simultaneously taken photos of different females show *A. mixta* (characters 7b, 8) (SCHRÖTER 2010: fig. 23) and *A. soneharai* (unpublished). A photo of observation no. 57902969 by Ruslan Nurkhanov from Lake Alakol' in Kazakhstan (45.98° N, 81.51° E) shows simultaneously both a male of *A. mixta* and a female of *A. soneharai* (Fig. 10), although the male (maybe a hybrid of some generation?) has unusually for *A. mixta* large dorso-posterior spots on the abdomen and bluish (but still transverse) centro-posterior spots. If this individual actually represented *A. mixta* or was a hybrid, this would be the third registered case, after Moscow and Alamedin Canyon, where both species were found together in the same locality.

Since *A. mixta* was not observed by VVO in Moscow and Moscow and Vladimir Provinces prior to 2021 and considering that *A. mixta* is steadily

expanding northwards in the British Isles and North Europe (KALKMAN *et al.* 2015), it is likely that the European range of this species in Russia is currently expanding to the north and east, overlapping the western range of *A. soneharai*.

Preliminary notes on variation of *Aeshna mixta* and *A. soneharai* over their ranges

Above we listed the important diagnostic characters and some additional characters different in individuals of both species from the Moscow area (Table 1). Caution was naturally needed to expand these results to the huge ranges of these species. To preliminarily evaluate the stability and importance of the characters of Table 1, we examined the same dataset of photographic observations in iNATURALIST (2021) and checked there all observations identified as *A. mixta* which existed to mid-November 2021. Some



Figure 9. A schematic map of distribution of *Aeshna mixta* (●) and *A. soneharai* (●) in the Old World as identified by us in photographic observations at the iNaturalist (2021) database, with data from SCHRÖTER (2010) and this paper added. Grey circles stand for photographs not showing diagnostic characters.

additional information was considered. As expected for such common and widespread species, both were found to be variable.

Aeshna mixta is a better studied species, overwhelmingly predominating over *A. soneharai* in iNaturalist observations, which is why it is no surprise that it exhibited considerable variation. In Western and Central Europe, the dorsal S2 pale mark in males varies from a short and broad yellow ‘nail’, as shown in the European guides (e.g., DIJKSTRA *et al.* 2020) to a narrow, rather yellowish or whitish (rarely bluish) nail produced caudad with a fine line, but not a broad blue stripe as in *A. soneharai*. Males from Western



Figure 10. A putative male of *Aeshna mixta* (left) and a female of *A. soneharai* (right) in one shot, south-western bank of Lake Alakol' south-eastern Kazakhstan, iNaturalist observation no. 57902969. Photo by courtesy of Ruslan Nurkhanov (18. viii.2020). Not to scale.

Asia and Kashmir exhibit some extending and bleaching of pale elements of the pattern. The S1 lateral pale area, lunular in *A. mixta* versus triangular in *A. soneharai* (character 6) is among the best and persistent character in most of the range. However, in individuals of *A. mixta* from the southern part of the range, this area tends to expand to occupy most of the S1. It nevertheless does not occupy the latero-ventral rib of the segment and leaves some of the brown ground colour there, which is not always well seen in photographs. It may look like a blunt triangle, but not adjacent to the tergite ventral rib, with a free blunt apex. Some observations of *A. mixta* show females with the pale elements of abdominal maculation being blue, but not as bright as in males. The character 4 of Table 1 hardly works in Western Europe since specimens of *A. mixta* often occur with double spots on the costal plates, as in *A. soneharai*.

Günther Fleck (pers. comm.) kindly informed us that, according to a sample of 50 individuals photographed *in vivo* by P. Juliand in the Ardèche département of France, some individuals of *A. mixta* occur with individual characters attributed in Table 1 to *A. soneharai*. This concerns the following characters: whitish male face (character 1), a narrow T-mark on the frons (character 2); an inner cell in the anal loop (character 3), a pair of blue spots and a minute median spot at S1 anterior margin in males (character 5); S1 lateral spots in some females almost like in *soneharai*, in males these spots often expanded as described above (character 6); the S2 mark in males completely or partially blue, sometimes barely tapering posteriorly (character 7a); bluish central dorsal spots in most individuals (part of character 8); posterior dorsal spots of variable size (character 9), bluish and not so small postero-ventral spots in most individuals (character 10); complete blue rings at abdominal segment joints (character 11); blue and/or medium-sized pale spots on S10 (character 12); relative cercus length overlapping to that specified for *A. soneharai* in Table 1 (character 13). The Ardèche region therefore obviously deserves a thorough study of occurrence and correlation of the characters from Table 1. Nevertheless, as judging by those photos, each exceptional individual remained identifiable as *A. mixta* by other characters of the complex. The semi-lunular or blunt triangular lateral spots on S1 (character 6) and narrow central dorsal spots on abdominal segments (character 8) remain the most reliable characters. iNaturalist

observations suggest that the same moderate occurrence of the ‘*soneharai*’ characters listed in Table 1 takes place also elsewhere in the Mediterranean and probably in Western Europe in general. A statistical analysis of occurrence of characters from Table 1 is impossible for occasional photos of that platform but would be most welcome for series of alive individuals, examined in hand in different regions.

Aeshna soneharai is rather uniform across the European part of Russia and Siberia and becomes more variable in the Far East of Russia and in Korea, while there is no observation from Japan at iNaturalist. Most individuals photographed show the same set of key characters as those from Moscow. There is a hardly noticeable trend of the abdominal maculation becoming smaller in the Far East, at least more variable in size. In some individuals, pale spots are whitish rather than blue. The upper surface of the frons in males, although not claimed as a diagnostic character in Table 1, is strongly yellowish in some individuals. One male from the Vladivostok environs (observation 9442502) has the S2 dorsal mark shortly triangular (as in *mixta*) but still saturated blue, in some others the blue stripe is strongly narrowed. The morphological characters are considered more reliable than coloration characters to discriminate the species. However, the male epiproct (character 14) may become more variable and just longer in the east of the *A. soneharai* range, e.g., in Primorye, in some specimens seemingly comprising ca 55–57% of the cercus length, as far as small photos taken at different angles could suggest. Good measurable illustrations in two books on Korean Odonata provided the epiproct length to comprise, again, 55% (KIM *et al.* 2020) and 57% (CHO 2021) of the cercus length, not yet reaching 62–65% exhibited by the Moscow specimens (Table 1, Fig. 3b).

The observation 99055491 by E. Khokhrina from Ulybino village in Novosibirsk Province (54.58° N, 82.93° E) shows a strange male with the S2 dorsal mark rather as in *A. mixta*, the centrodorsal abdominal spots as large and roundish as in *A. soneharai* but whitish, and a relatively long epiproct. Other specimens examined by the authors or photographed in iNaturalist from Novosibirsk Province were like *A. soneharai* from Moscow Province. The individual in question could also be a hybrid between these two species considered, although typical *A. mixta* have not yet been observed in that area.

Summarised, the complexes of most important characters of the two species claimed, *A. mixta* and *A. soneharai*, recognised in Moscow specimens (Table 1) work rather well across the entire Palaearctic but there exist exceptions with respect to each individual characters, even morphological ones.

The phenotype of the specimens from Japan (SUGIMURA *et al.* 2001; AOKI 2021 and some internet sources) considerably differs from that of the continental specimens with much smaller – up to complete reduction – but still rounded central dorsal spots on abdominal segments. As may be judged from photos in Internet, the overall extent of the pale maculation is quite variable in Japanese specimens. A question arises if the continental populations may represent an undescribed subspecies of *A. soneharai* characterised by large central dorsal spots on abdominal segments in males, which we leave to further quantitative analysis of the size of these spots in specimens from all over the range of *A. soneharai*.

Putative behavioural differences of *Aeshna mixta* and *A. soneharai*

Aeshna mixta is well-known for its ability to make long migrations, often in swarms, especially during its maturation period in the mountains (SAMRAOUI *et al.* 1998; KALKMAN *et al.* 2015). According to our observations in the Caucasus, a remarkable feature of its behaviour is clustering of individuals into foraging swarms, mostly formed by pre-breeding individuals and occasionally together with *A. affinis*. Dozens to hundreds of individuals move slowly over larger gaps in arboreal vegetation such as forest glades or roads of river valleys, sometimes near conspicuous large objects like prominent trees, cliffs, houses *etc.* There is a permanent turnover of individuals either flying or resting on trees (KOSTERIN & SOLOVYEV 2017). The same concentrating and swarming of numerous immature *A. mixta* in mountain gorges was also observed by OEK in two remote regions situated much to the south-east of the Caucasus, *viz.* the southern foothills of the Tyshkantau Massif in the southern Dzungarian Alatau Mts, south-eastern Kazakhstan (KOSTERIN & BORISOV 2010) and in High Zagros Mts in Lorestan Province of Iran (KOSTERIN & AHMADI 2018). The same phenomenon was observed by SAMRAOUI *et al.* (1998) in Algeria. Curiously, the authors never observed such swarms in the territories of Russia occupied by *A. soneharai*, *viz.* in Moscow and Vladimir Provinces and more easterly in the European part of

Russia, in Ural, Siberia, and the Far East. Remarkably, according to observations by VVO, in the southern Far East of Russia several species of Aeshnidae usually form mixed foraging swarms in the evening twilight, which often include also *Somatochlora* and larger *Sympetrum* spp. (ONISHKO & KOSTERIN 2021), but never *A. soneharai*. It seems that *A. soneharai* does not share the swarming behaviour of *A. mixta* and could be a less active flier. Therefore, one could speculate that it also does not share the migratory abilities of its close relative. The fact that no copulae of *A. mixta* were seen 2021 in Moscow, while those of *A. soneharai* were frequent, might indicate that while the latter was at its regular breeding habitats, *A. mixta* could have been represented by vagrant individuals dispersed from elsewhere, which for some reason did not find the examined localities useful for breeding.

Analysis of observations in iNATURALIST (2021) provided several photographic records showing terrestrial oviposition of *A. mixta* into dry stems (observations 32827653, 41907682, 57368444 from England, 37203645 from Germany, 33949727, 38550602 from Italy). On the other hand, VVO and OEK have observed *A. soneharai* females ovipositing into water only. This may indicate more flexible oviposition behaviour in *A. mixta* than in *A. soneharai*.

General discussion

The subject of this paper is a case when geographically distant researchers for a long time used to assume local individuals to belong to the same well-known and broadly distributed species, thereby overlooking differences. Firstly, this concerns the Siberian odonatological school founded by Boris Fedorovitsch Belyshev, whose representatives used to be familiar with specimens from Ural, Siberia (BELYSHEV 1973; BELYSHEV *et al.* 1989; HARITONOV & EREMINA 2008; MALIKOVA & KOSTERIN 2019), Central Asia (BELYSHEV *et al.* 1989; BORISOV & HARITONOV 2008), and the Russian Far East (MALIKOVA 1995; MALIKOVA & KOSTERIN 2019). They regarded all *mixta*-like individuals to be genuine *A. mixta* while they in fact pertained to a different taxon. ASAHINA (1988) recognised the specimens from East Asia, including Japan, the Far East of Russia, and Northeast China, to represent a taxon different from European *A. mixta*. He regarded this taxon as the eastern subspecies of the latter and described it as *A. mixta soneharai*. It

was necessary to encounter both taxa in sympatry in Moscow and Moscow Province in 2021 to suspect them to be two distinct species.

Although inferred from Moscow individuals, the two sets of diagnostic characters (Table 1), related to both morphology and colour pattern, facilitate the separation of *A. mixta* and *A. soneharai* over their vast ranges. However, in huge territories where only one species of the two is found and there is no sympatry, the phenotypes of either species 'relax', so that several individuals may occur, with some characters falling out of the 'typical' complex or showing unclear character formation. Nevertheless, with very few exceptions, identification remains reliable with respect to the majority of characters. The pattern of individual and geographical variation in both species remains to be better assessed throughout their range. The above supposed 'relaxation' of diagnostic characters of both species in regions like the above-mentioned southern France, Primorye, or Korea, distant from the area of their sympatry, should be thoroughly documented. We hope this study will trigger gathering of data from all over the vast range of what was hitherto presumed to be the single species, *A. mixta*, on distribution, individual and geographical variation of the two species, as well as clarification of their putative differences in behaviour.

The *COI* sequence of the *A. soneharai* specimens from Moscow and Japan (sequenced by KARUBE *et al.* 2012), separated by ca 7500 km, are strikingly almost identical (one nucleotide substitution), thus evidencing close relatedness of these specimens with such remote provenances. At the same time, both mitochondrial sequences of the Moscow specimens of *A. soneharai* differed from those of the specimens of *A. mixta* from the same localities in Moscow and elsewhere. The diagnostic nucleotide positions comprised 1 % of all positions in the *COI* fragment sequenced while the overall genetic distance between the two species varied as 1.7–2.88 %; in the case of *COII* the genetic distance was 2.58 %. The unresolved position of specimens from Bosnia and Herzegovina and the resulting poor clustering of *A. mixta* in the tree based on the *COI* fragment deserves further phylogeographic study of *A. mixta*, hopefully with involvement of more target sequences. As such it does not weaken our conclusion that *A. soneharai* is a separate species. It should be noted that the *COI* barcoding fragment involved was shown to not

always resolve even distinct species of Odonata (Dow *et al.* 2019), while the mitochondrial DNA phylogeny can be unpredictably affected by mitochondrial introgression (BALLARD & WHITLOCK 2004) and, in insects including Odonata, by such selective factor as the optional endosymbiont *Wolbachia* infection (DENG *et al.* 2021). The nuclear *ITS2* sequence, a marker used for DNA taxonomy and barcoding, provided no variation in the specimens we tested, so involvement into molecular analysis of more (and hopefully more variable) nuclear sequences is highly desirable. Anyway, the cautious conclusion of this paper, of the existence of two distinct species, previously lumped under the name *Aeshna mixta auct.*, is first of all based on two distinct and concordant complexes of morphological and coloration characters observed in sympatry in Moscow (Table 1, Figs 1–3, 6), appeared to be steady enough over vast territories (Fig. 9) and is supported by both mitochondrial sequences analysed (Figs 7, 8), although not so by the nuclear sequence. This situation can hardly be interpreted otherwise, because of (i) too many unrelated characters for polymorphism for alleles of one gene with pleiotropic effect, (ii) the striking proximity, almost identity of *COI* sequences of *soneharai* from Japan and Moscow, and (iii) too scarce introgression (at the level usually observed for any related Odonata species) for two insufficiently diverged gene pools ('semispecies') met in a secondary contact zone. An alternative but hardly probable interpretation of the observed scarce introgression could be that the *mixta*-type was found in the Moscow area at the beginning of its expanding there, so that the two putative 'semispecies' just had no time to mix. If so, *soneharai* could be interpreted as a genetically homogeneous clade within a highly differentiated species *A. mixta*, which had pre-occupied the Moscow area long before of the appearance of *mixta s. str.* However, the photographic data of occurrence of both species (Fig. 9), although still insufficiently dense, may be interpreted so that the contact zone is actually as wide as *ca* 1 300 km between Moscow and the Ural.

The status of *Aeshna lucia* still remains unsolved. No indication of a dragonfly that comes close to its holotype as described by NEEDHAM (1930) has appeared for almost a century. Synonymisation of *A. lucia* with *A. mixta* by ASAHINA (1988) was very cautious and based on the structure of the cerci figured in the original description and venation – to the date of examination

of the holotype by Asahina, only both right wings remained from it. BELY-SHEV & DOSHIDORZHI (1958) tentatively attributed a female aeshnid from Mongolia to *A. lucia*, considering the black face as the main diagnostic character, but without having access to the original description. Elena I. Malikova (pers. comm.; KOSTERIN 2004) re-examined that specimen and identified it as *Aeshna juncea* (Linnaeus, 1758). KOSTERIN (2004) and BELEVICH (2005) still considered the black face the main diagnostic character of *A. lucia* and supposed that the face may have darkened *post-mortem*. Based solely on this character, BELEVICH (2005) upheld the synonymy of *A. lucia* with *A. mixta* as suggested by ASAHINA (1988), disregarding other characters mentioned by NEEDHAM (1930). However, in view of the new insight presented here it should be noted that Beijing, the type locality of *A. lucia*, is within the range of *A. soneharai* and very far from that of *A. mixta*. In the figure by NEEDHAM (1930: plate viii, 11), the epiproct is depicted slightly longer than half of the cercus length and S10 bears large and distinct pale spots, which also fits *A. soneharai* rather than *A. mixta* (Table 1). Thus, if *A. lucia* is indeed a synonym of *A. soneharai* rather than of *A. mixta*, the name *lucia* Needham, 1930 is older than *soneharai* Asahina, 1988 and would have a priority if the synonymy can be proved. However, the unique combination of other characters of the holotype of *A. lucia* described in detail by NEEDHAM (1930) does not fit any known species of *Aeshna*, which is why we consider it more sensible to regard it as a dubious species. Of course, if such a distinct species described from Beijing existed in fact, it would most probably have been encountered again during almost a century that passed since its description. Nevertheless, at present there is not enough evidence to claim the above synonymy. This situation will not change unless 'the true *A. lucia*' is rediscovered, which is not very likely.

In conclusion, we propose to list *A. lucia* no longer as a synonym of *A. mixta* and to include it in world catalogues as a full but doubtful species, *Aeshna lucia* Needham, 1930.

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