The species-group taxa in the Holarctic genus *Cordulia*: a study in nomenclature and genetic differentiation (Odonata: Corduliidae)

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ABSTRACT

Two *Cordulia* species have been described so far, the Eurasian *C. aenea* and the North American vicariant *C. shurtleffii*. The names *amurensis*, *tatrica*, *turfosa*, *laubmanni*, *linaenea* and *aeneaturfosa* are available in the synonymy of *C. aenea*. Out of these, only the name *amurensis* is deemed to denote a separate taxon, which is currently regarded as a weakly defined Far East subspecies. A molecular analysis of nuclear ITS I sequences of specimens representing all three taxa *aenea*, *amurensis* and *shurtleffii* shows a clear differentiation which suggests a recognition of three separate taxa with full biological species rank. The morphological and distributional differentiation between *C. amurensis* and *C. aenea* requires further investigation. The molecular approach based on the variability of nuclear ITS I sequences appears promising for further taxonomic studies within dragonflies.

INTRODUCTION

From the current state of taxonomy (e.g. Davies & Tobin 1985; Tsuda 1991; Bridges 1994; Steinmann 1997) the genus *Cordulia* Leach, 1815 contains two species, the Palaearctic *C. aenea* (Linnaeus, 1758) and its Nearctic vicariant *C. shurtleffii* Scudder, 1866. *C. aenea* is presently split into two subspecies. The nominotypical ssp. is distributed between western Europe and Siberia, whereas ssp. *amurensis* Selys, 1887 occurs in the Far East. However, this taxonomic approach is far away from being uncontested. A comparison of Siberian *aenea* and *amurensis* showed a surprisingly weak differentiation between both taxa, being limited to slight differences in their measurements only (Belyshev 1956). The relationship between *aenea* and *shurtleffii* has been discussed in terms of conspecificity (Belyshev 1973). Some confusion about the type of *aenea* demonstrates the necessity of a well balanced understanding of the taxonomy within the genus *Cordulia*. Linnaeus (1758) introduced the name *aenea* on basis of a heterogenous type series containing two different species, currently known as *C. aenea* and *Somatochlora flavomaculata*

(Vander Linden, 1825). Based on arguments given by McLachlan (1898), Fraser (1956) designated the *flavomaculata* specimen to be the lectotype of *aenea*, with the consequence that the senior name *aenea* now denotes the *Somatochlora* species, and that the *Cordulia* species is in need of another name. Two such names have been introduced: these are *C. linaenea* Fraser, 1956 and *C. aeneaturfosa* Buchholz, 1967. Both names are invalid due to their disregard of priority of available synonyms but, without a better insight into the relationships between all *Cordulia* taxa, it is not possible to define the oldest synonym of *aenea*. Meanwhile Jödicke & van Tol (2003) proposed the replacement of the lectotype of *L. aenea* with a newly designated lectotype. Although a nomenclatural clarification would make it superfluous to search for the oldest synonym, we considered it helpful to provide a critical review of all specific names introduced into the genus *Cordulia*. In the present paper we also test the taxonomic assignations of *aenea*, *amurensis* and *shurtleffii* with the new methods of molecular genetics.

MATERIAL AND METHODS

All specific *Cordulia* names were checked on the basis of the International Code of Zoological Nomenclature (ICZN) (International Commission on Zoological Nomenclature 1999). The current taxonomic approach is based on a compilation of opinions published in the relevant literature.

For the genetic analysis, specimens of *C. aenea aenea*, *C. aenea amurensis* and *C. shurtleffii* were taken from the entire ranges of all three forms (Table 1). *Somatochlora metallica* (Vander Linden, 1825), *S. flavomaculata* and *S. alpestris* (Selys, 1840) served as outgroup species (Table 1). Specimens were preserved in 95% ethanol and stored at room temperature. Voucher specimens used for DNA analysis were deposited in the tissue collection of the Zoologisches Forschungs-institut und Museum A. Koenig, Bonn.

The genetic analysis relied on the recorded variability of the nuclear Internal Transcribed Spacer I (ITS I) found between the nuclear 18 S and 5.8 S ribosomal subunits (Hillis & Dixon 1991). The ITS I as well as the ITS II region within the nuclear genome are well known for their hypervariability and have been used in phylogeographic and phylogenetic studies (Hillis & Dixon 1991; Weekers et al. 2001; Pilgrim et al. 2002; Samraoui et al. 2002, 2003). For DNA extraction, thoracic muscle tissue was excised from each specimen and DNA was extracted using the Nucleospin®Tissue Kit (Macherey-Nagel). PCR amplifications of ITS I coding DNA was achieved using the primer pair ITS1 for: 5'TAGAGGAAG TAAAAGTCG3' and ITS1rev: 5'GAATCGTGGGCTGCAAT3' (Weekers et al. 2001). Both primers are located inside the flanking rRNA coding genes, and therefore amplify the entire ITS I sequence. PCR products were purified and cleaned of unincorporated primers and nucleotides with the Nucleospin®Extract Kit (Machery-Nagel). PCR products were subsequently sequenced using the ABI Prism Big Dye™ ready mix (Applied Biosystems) according to manufacturer protocols. Cycle sequencing products were separated and visualized on an ABI 377 XL automated sequencer (Applied Biosystems). PCR products were sequenced from

Table 1. Origin of specimens analysed and species assignation.
ID #: identification number; AC #: GenBank accession number.

Country	Collecting site	ID #	AC #	Species
S Great Britain	Hampshire, 51°18'N, 0°53'W	1	AY274516	*aenea*
		2	AY274517	
		3	AY274518	
		4	AY274519	
W Germany	Börstel, 52°38'N, 7°42'E	5	AY274520	
		6	AY274521	
		7	AY274522	
E Poland	Dorohusk, 51°10'N, 23°48'E	8	AY274523	
	Tomaszów Lubelski, 50°27'N, 23°25'E	9	AY274524	
		10	AY274525	
C Russia	Novosibirsk, Siberia, 55°05'N, 82°50'E	11	AY274526	
		12	AY274527	
		13	AY274528	
Switzerland	Zürich, 47°14'N, 08°44'E	14	AY274529	
		15	AY274530	
Sweden	Umeå, 63°49'N, 20°14'E	16	AY274531	
		17	AY274532	
		18	AY274533	
E Russia	Blagoveshchensk, Amur prov., 50°16'N, 127°32'E	19	AY274534	*amurensis*
		20	AY274535	
		21	AY274536	
Japan	Pon-Numa, 43°05'N, 144°25'E	22	AY274537	
		23	AY274538	
		24	AY274539	
E Russia	Tommot, Yakutia, 59°00'40"N, 126°11'E	25	AY274540	
		26	AY274541	
N USA	Minnesota, 47°24'N, 91°15'W	27	AY274542	*shurtleffii*
		28	AY274543	
NE USA	Maine, 46°98'N, 69°28'W	29	AY274544	
		30	AY274545	
W Canada	British Columbia, 56°46'N, 127°10'W	31	AY274546	
		32	AY274547	
		33	AY274548	
France	Massive Centrale	35	AY274551	*flavomaculata*
Austria	Lower Austria, Gföhl	36	AY274550	*metallica*
	Tirol, Zillertal	37	AY274552	*alpestris*
		38	AY274553	

both strands and complete sequences assembled using BioEdit (Hall 1999). Sequences were deposited in GenBank under the Accession numbers AY274516AY274553. Sequences were aligned using Clustal X (Higgins et al. 1996) employing the default alignment parameters. The alignment was visually checked and corrected for obvious misaligned positions.

Sequence relationships were analysed with the neighbor-joining method (Saitou & Nei 1987) using simple *p*-distances in PAUP (Swofford 2002) and with a parsimony network using the software TCS (Templeton et al. 1992; Clement et al. 2000).

NOMENCLATURAL ANALYSIS
with annotations on current taxonomy

The Palaearctic *Cordulia aenea*

A comparison between the world systematic catalogues of dragonflies (Davies & Tobin 1985: 62f; Tsuda 1991: 132; Bridges 1994: VIII.18; Steinmann 1997: 255f) reveals a remarkable disagreement in the treatment and taxonomic evaluation of the names that have been introduced into the synonymy of *C. aenea*. This is why all these names are again scrutinized under nomenclatural and taxonomic aspects. The names are chronologically ordered and given in the original version.

Libellula aenea Linnaeus, 1758

Type: originally three syntype specimens. ♂ no. 768: currently assigned to *Somatochlora flavomaculata*; ♂, ♀ no. 769: currently assigned to *Cordulia aenea*, ♂ lost before 1898; the remaining specimens now deposited in the Linnean coll., Linnean Society of London. Nos 768/769 refer to the enumeration in 'Fauna Suecica' (Linnaeus 1746). ♂ 768: lectotype designation by Fraser (1956); ♀ 769: proposed as newly designated lectotype (Jödicke & van Tol 2003).
Locus typicus: Europe.

The name *aenea* (Linnaeus 1758: 544) has been in prevailing use so far to denote the *Cordulia* species. However, McLachlan (1898) analysed the species description and took the contradictory view that Linnaeus intended to denote the *Somatochlora* species. Later Fraser (1956) provoked a nomenclatural dilemma, when he concurred with McLachlan's interpretation and indicated the *Somatochlora* male as the 'type'. This act has to be taken as the lectotype designation of *L. aenea* (Art. 74.5 ICZN), with the consequence that the name *flavomaculata* would be a junior synonym of *aenea* and that the *Cordulia* would be an unnamed species. Although it has been shown that the arguments given by McLachlan (1898) and Fraser (1956) are a misinterpretation of Linnaeus' original intention (Longfield 1957; Jödicke & van Tol 2003), Fraser's lectotype designation had to be regarded as binding (Art. 74.1.1 ICZN). With the purpose to serve for nomenclatural stability, Jödicke & van Tol (2003) therefore asked the International Commission on Zoological Nomenclature to replace Fraser's lectotype in favour of Linnaeus' ♀ 769 as the newly designated lectotype.

Cordulia aenea variété *amurensis* Selys, 1887

Type: a syntype series of ♂ and ♀, presumably still deposited in the Selysian coll., Institut Royal de Sciences Naturelles, Brussels.

Locus typicus: Pokrofka (= Pokrovka), Amur region, Russia.

When Selys (1887: 51) introduced the name *amurensis*, he explicitly stated that the Far East specimens did not merit subspecific rank: "C'est plutôt une variété locale *amurensis* si l'on veut, qu'une véritable race." Accordingly he did not include *amurensis* in his index of newly described species and subspecies (p. 85). Regarding Art. 45.6.4.1 ICZN, the name nevertheless cannot be deemed to be infrasubspecific, since *amurensis* was adopted for the first time as the valid name of an easterly subspecies by Bianchi (1904: 758).

In the Siberian and Japanese literature, *C. a. amurensis* is broadly accepted as a 'good' subspecies. This fact appears somewhat curious given the poor definition of characters of this taxon. The original description stresses (a) the smaller measurements and (b) the absence of amber spots on the wing bases of the series from the Amur River.

(a) Belyshev (1956) compared measurements of his specimens from West and Central Siberia, Transbaikalia and the Far East with those provided by Selys (1840, 1887) and Bartenef (1912, 1914). Besides a critical note on obviously different methods used to measure the length of the abdomen, he confirmed that specimens from eastern Siberia are smaller than those from western Siberia and Europe. He concluded from this fact that the separation of *amurensis* on an subspecific level is justified.

(b) Previously, Bartenef (1912) did not assign a series from Transbaikalia to *C. a. amurensis* since all specimens had distinct amber on the wings. Belyshev (1956) analysed the presence or absence of amber on the base of the hindwings and came to the conclusion that there is only a tendency from west to east for a reduction in the intensity of amber markings, but not a defined geographical distribution of the clear wing base. He therefore rejected the clear wing base as a reliable subspecific character.

Summarizing the assessment of Belyshev (1956, and subsequent papers), *C. a. amurensis* in eastern Siberia differs from the typical subspecies only in its smaller size. In an identification key by Belyshev (1973) all specimens distributed easterly from a line from Lake Baikal to the Yana River and with a hindwing length up to 30 mm in males and 31 mm in females are assigned to ssp. *amurensis*. The typical ssp. *aenea* is defined to be distributed in the remaining part of the range, with a hindwing length not less than 32 mm. This conception has been maintained by subsequent Russian authors up to now, although the separation in two different subspecies has been questioned by some authors more recently (O. Kosterin pers. comm.). Symptomatic of a currently changing conception is a recent paper by Ivanov (2002) in which a specimen from Sakhalin is not assigned to *C. a. amurensis*.

Regarding its occurrence in Japan, Asahina (1938) still listed *C. aenea* without its subspecific name. Asahina (1949) was most probably the first Japanese author to use the name *C. a. amurensis*. All subsequent authors obviously followed his lead. Nobody, since Asahina, has questioned the subspecific character of the Japanese population (K. Inoue and H. Ubukata pers. comm.). Okudaira et al. (2001)

demonstrate a broad variety in the expression of amber wing spots in the Japanese population and give measurements that partially overlap with those provided by Schmidt (1929) for Europe.

Cordulia aenea var. *tatrica* Dziędzielewicz, 1902

Type: ♂, leg. A. Wierzejski; deposited in Muzeum Fizjograficzne, Cracow.
Locus typicus: Toporowy lake, Tatra Mountains, Poland.

In contrast to typical *aenea*, this male has two oblong, yellow spots on the frons. Dziędzielewicz (1902: 95) assumed that the variety might be a new species. This assumption makes the name *tatrica* available (Art. 45.6.4 ICZN). More specimens from the type locality and also from outside the Tatra Mountains have been collected by Fudakowski (1930). This author considered *tatrica* an infrasubspecific form, since specimens with yellow spots on the frons co-occurred with normal specimens and no other distinguishing characters were to be seen.

The preface by Dziędzielewicz (1902) is dated from May 1902, but we are not able to verify the date of publication. So the precedence of *tatrica* or *turfosa* (see below) remains an open question.

Cordulia aenea turfosa Förster, 1902
Cordulia aenea-turfosa Förster, 1902 (not available)

Type: ♂, leg. 24 July 1898; deposited in Museum of Zoology, University of Michigan (Garrison et al. 2003).
Locus typicus: Nonnmattweiher in the Schwarzwald (Black Forest), SW Germany.

Förster (1902) introduced a new taxon on the basis of a single male which he caught at a *Sphagnum* bog. The specimen was slightly smaller than those from oxbows in the nearby Upper Rhine valley. Its anal appendages were said to resemble those of the Nearctic *C. shurtleffii*. The subspecific name was spelled in two different ways in the same paper: as a separate word "*turfosa*" and linked to the specific name by means of a hyphen "*aenea-turfosa*". The latter spelling would require a correction by removing the hyphen (Art. 32.5.2.3 ICZN), leading to the binomen *C. aeneaturfosa*. Förster undoubtedly intended to establish a new sub-species, since he wrote about "*Cord. aenea* L. und ihre Torfrasse". Accordingly, on the label of the holotype Förster wrote "Subrasse turfosa m[ihi]" (Garrison et al. 2003). In this respect only the trinomen *C. aenea turfosa* can be the correct original spelling (cf. also Jurzitza 1969: 191). Thus the spelling *aenea-turfosa* has to be regarded as the incorrect version of multiple original spellings (Art. 19.3 ICZN).

The name *turfosa* is in current use. Davies & Tobin (1985), Bridges (1994) and Steinmann (1997) list *turfosa* as a valid subspecific name, in contrast to Tsuda (1991) who considers *turfosa* an infrasubspecific variety. However, the subspecific rank of *turfosa* has never been confirmed. In present view the male in question reflected the typical modification of a dystrophic habitat rather than an own taxon.

Cordulia aenea laubmanni nov. ssp. Götz, 1923

Type: ♂, leg. 6 July 1922; deposited in coll. Götz, now probably in the Bayerische Staatssammlung, Munich.

Locus typicus: Korbsee near Markt Oberdorf, subalpine Bavaria, SE Germany.

Specimens from the Allgäu region were said to be smaller, the males with a more notched inferior anal appendage "deutlich gekerbt", and with a more extensive, grey rear of the membranula (Götz 1923). Although neither Schmidt (1929) nor succeeding authors took *laubmanni* into consideration as a distinct taxon, it is still listed with subspecific rank by Davies & Tobin (1985), Tsuda (1991) and Bridges (1994). From the present taxonomic view no distinct subspecies occurs in that region. Thus the name *laubmanni* is herewith synonymized with *aenea*.

Cordulia aenea var. *brachycauda* n. var. Görtler, 1948 (not available)
Cordulia aenea var. *longicauda* n. var. Görtler, 1948 (not available)

Type *brachycauda*: ♂ leg. 19 May 1946, coll. Görtler, present deposition unknown. Locus typicus: Čelákovice, Czech Republic.
Type *longicauda*: ♂ leg. May 1947, coll. Görtz, present deposition unknown. Locus typicus: Praha-Chuchle, Czech Republic.

Görtler (1948) described and drew one male with an extremely short and another male with an elongated inferior appendage. The descriptions unambiguously show that both names were proposed for individual aberrations, thus being infrasubspecific names (Arts 45.6.1 and 45.6.4 ICZN).

Cordulia linaenea nov. nom. Fraser, 1956

Type: *aenea* ♂ no. 769 in the Linnean coll. (see above).

Fraser (1956) introduced this name in consequence of his lectotype designation of *aenea* in favour of the *Somatochlora* specimen (s. above). Due to Fraser's disregard of priority (Longfield 1957), the name was never accepted.

Cordulia aenea amurensis ab. *selysi* Belyshev, 1956 (not available)

This aberration covers all specimens from the range of *C. aenea amurensis* that lack a yellow spot on the wing bases. Such specimens are deposited in the Belyshev coll., Institute of Systematics and Ecology of Animals, Academy of Sciences, Novosibirsk.

Because Belyshev (1956: 190) combined the new name with a trinomen and defined it to denote an aberration, *selysi* undoubtly is infrasubspecific (Arts 45.5 and 45.6.2 ICZN). This also conforms with later interpretations (Belyshev & Kurenzov 1964; Belyshev 1964, 1966, 1973).

Cordulia aeneaturfosa Buchholz, 1967

Buchholz (1967) knew that Fraser's new replacement name *linaenea* (see above) would be a disregard of priority. He therefore suggested the usage of the name *aeneaturfosa* which he attributed to Förster (1902). He overlooked the correct original spelling *turfosa* (see above), so he cannot be regarded as First Reviser of Förster's multiple spellings (Art. 24.2.3 ICZN). Removing the hyphen in the incorrect version *aenea-turfosa*, Buchholz created a new name which has to be attributed to his own authorship (Art. 33.2.3 ICZN).

The Nearctic *Cordulia shurtleffii*

Cordulia bifurcata Hagen, 1861 (not available)

Specimens in the Selys collection were labelled under the provisional name *bifurcata* (see Selys 1871, 1878). When Hagen (1861: 137) introduced *bifurcata* to the North American fauna, he gave no description (cf. also Hagen 1867; Selys 1871). Consequently (Art. 12.1 ICZN), *bifurcata* is a nomen nudum.

Cordulia shurtleffii Scudder, 1866

Type: two syntype ♂, leg 11 and 25 August 1862; deposition presumably in the Museum of Comparative Zoology, Harvard Univerity (R. Garrison in litt.).
Locus typicus: Hermit Lake, White Mountains of New Hampshire, USA.
   The name *C. shurtleffii* (Scudder 1866: 217) is valid in its original version. Selys (1878) used the emended version *shurtleffi*, with only one -*i* in the suffix, for the first time. This became the general spelling for a long time. Despite the prevailing use, *shurtleffi* is an incorrect subsequent spelling which needs to be corrected (Art. 33.4 ICZN). Needham et al. (2000) were the first to return to the original spelling, following a correction by J. van Tol (in litt.). Other incorrect subsequent spellings are 'schurtleffii' (Selys 1871) or 'shurtletti' (Belyshev 1966) that do not constitute availability (Art. 33.3 ICZN).
   All Odonata world catalogues treat *C. shurtleffii* with full specific rank. On the other hand, Selys (1971) pointed out the morphological similarity between *C. aenea* and *C. shurtleffii*. Needham's observations on the biology of *C. shurtleffii* (Needham & Betten 1901) demonstrated also an ecological similarity relating to habitat selection and phenology. Förster (1902) considered the Palaearctic and Nearctic populations to be closely related, the subspecies *turfosa* being closer to *shurtleffii* than to typical *aenea*. Also Belyshev (1973) discussed the relationship between *C. shurtleffii* and *C. aenea*. Interpreting their morphological differences, he proposed a differentiation on the subspecies level.

GENETIC ANALYSIS: RESULTS

All three forms – *aenea, amurensis* and *shurtleffii* – were represented by multiple specimens from different collection localities. The aligned ITS I sequences contained 308 nucleotides. The *p*-distances were in the range of $d_p = 0.0\text{-}0.05$ within the genus *Cordulia*. The complete data set contained 76 parsimony-informative characters. Without outgroup taxa, the number of parsimony-informative characters dropped to 13, with gaps counting as missing characters. In total, we found 20 different haplotypes within the genus *Cordulia*.
   Haplotypes of *C. shurtleffii* shared seven autapomorphic plus six plesiomorphic character states compared to the outgroup taxa. Haplotypes of *C. aenea* showed one autapomorphic and four plesiomorphic character states, and haplotypes of *C. amurensis* shared two autapomorphic character states. These characters appeared fixed for assigned phenotypes. Remaining site variability was scattered
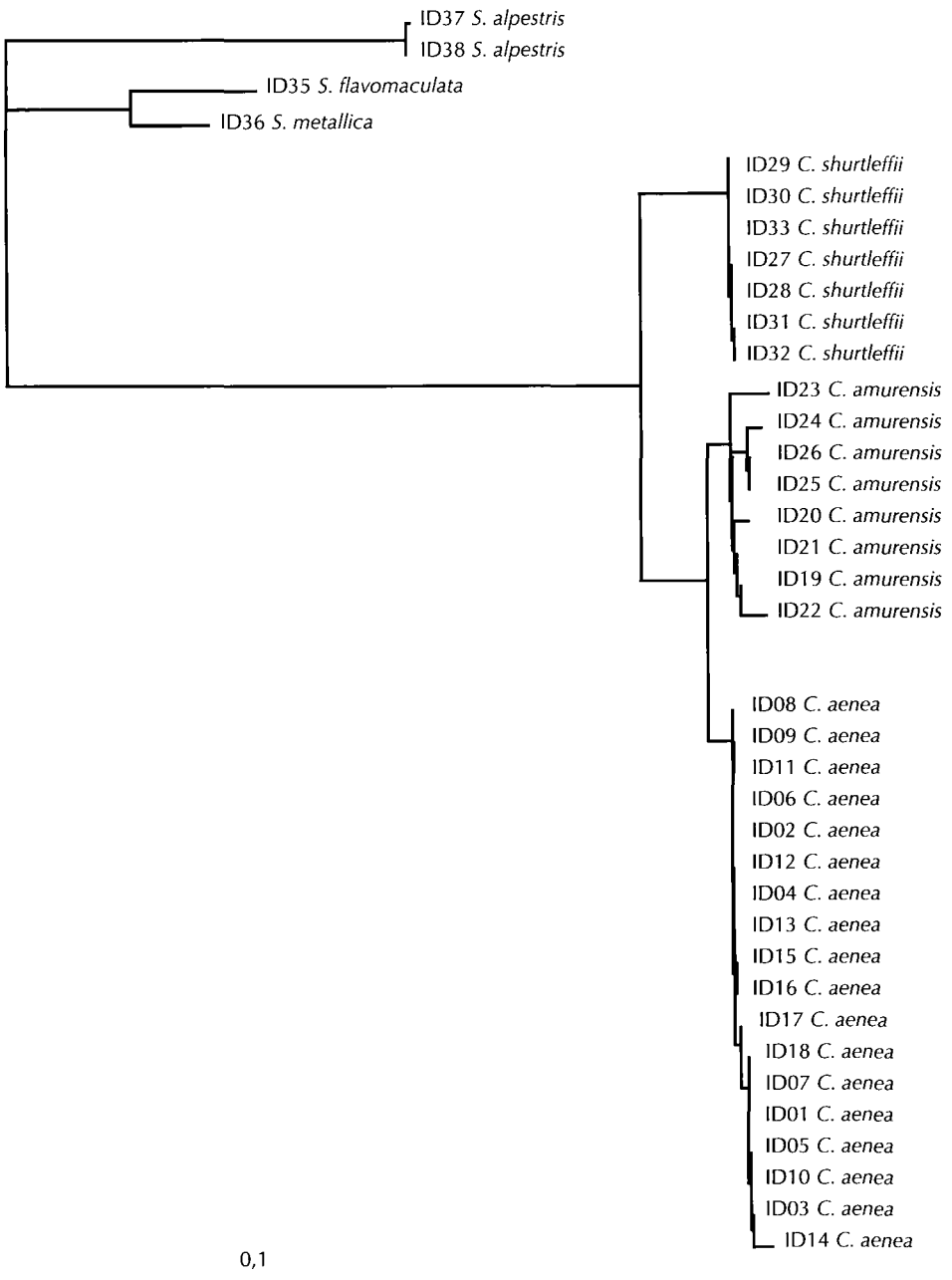
Figure 1: Neighbor-joining tree based on *p*-distances. The ITS sequences of *Cordulia aenea*, *C. amurensis* and *C. shurtleffii* have been rooted with ITS sequences of *Somatochlora alpestris*, *S. flavomaculata* and *S. metallica*. Representatives of *aenea*, *amurensis* and *shurtleffii* form three distinct monophyletic clades.

throughout the sequences without apparent phylogenetic information. The ITS tree using NJ-reconstructions and p-distances clearly separated all three forms (Fig. 1). A parsimony network reconstruction confirmed the results of the distance approach (Fig. 2). Haplotypes of *C. shurtleffii*, *C. aenea* and *C. amurensis* formed separate clades. The haplotype network did not suggest a simple haplotype differentiation by distance within *C. aenea* and *C. amurensis*. For example, *C. aenea* ITS haplotypes of Central Europe are not separated against haplotypes from Scandinavia or Siberia. In addition, the parsimony network reconstruction showed two major haplotypes within *C. aenea* (Fig. 2) which are found in England (ID01, 03), Germany (ID07), Poland (ID10), and England (ID02, 04), Germany (ID06), Siberia (ID11, 12, 13) and Sweden (ID17).

In summary, although there was variability even within *C. shurtleffii*, *C. aenea* and *C. amurensis*, all three forms displayed autapomorphic sites and were well separated in haplotype networks based on the ITS I sequences. It appeared that *C. shurtleffii*, *C. aenea* and *C. amurensis* haplotypes have reached the state of monophyly.

DISCUSSION

Within its huge range, *Cordulia aenea* shows some variability, not only in its mean size, but also in colouring and structure. Regarding the colour traits, yellow spots on the frons have been mentioned for specimens from Poland (Fudakowski 1930) and Turkey (Schneider 1986). The amber colouration of the basal wing area tends to be reduced in the Far East (Belyshev 1956) but also in Turkey (Schneider 1986). Götz (1923) referred to a Bavarian specimen with a different colouration of its membranula. Reports on structural differences are restricted to the male anal appendages (Förster 1902; Götz 1923; Görtler 1948); there are no comparative studies on the penile organ. The aforementioned variability partly led to the intro-duction of new names, but three of them – *brachycauda*, *longicauda* and *selysi* – are infrasubspecific and therefore not available. The others – *tatrica*, *turfosa* and *laubmanni* – do not denote valid subspecies, which makes them available only in the synonymy of *aenea*. Only *amurensis* is established as an subspecies of *aenea* although reliable discriminating characters are unknown. The Nearctic *C. shurtleffii* resembles *C. aenea* in morphological and ecological respect. Due to their allopatric ranges it is impossible so far to judge their taxonomic relationship; a potential conspecificity cannot be excluded (Belyshev 1973).

The genetic analysis shows the relationships of *C. aenea*, *C. amurensis* and *C. shurtleffii* in another light. All three forms are genetically clearly separated in the way that haplotypes achieved monophyly for each phenotype. In other words, they all display exclusive sets of genetic variability (Avise 2000), without signs of introgression of haplotypes from one form to the other. This genetic separation does not seem to be maintained solely by allopatric distribution. *C. aenea* and *C. amurensis* most likely do have a contact zone in the East Asian area. The clear separation of *amurensis* from haplotypes of *aenea* suggests a valid species status of both forms, which appears also true for *shurtleffii*.
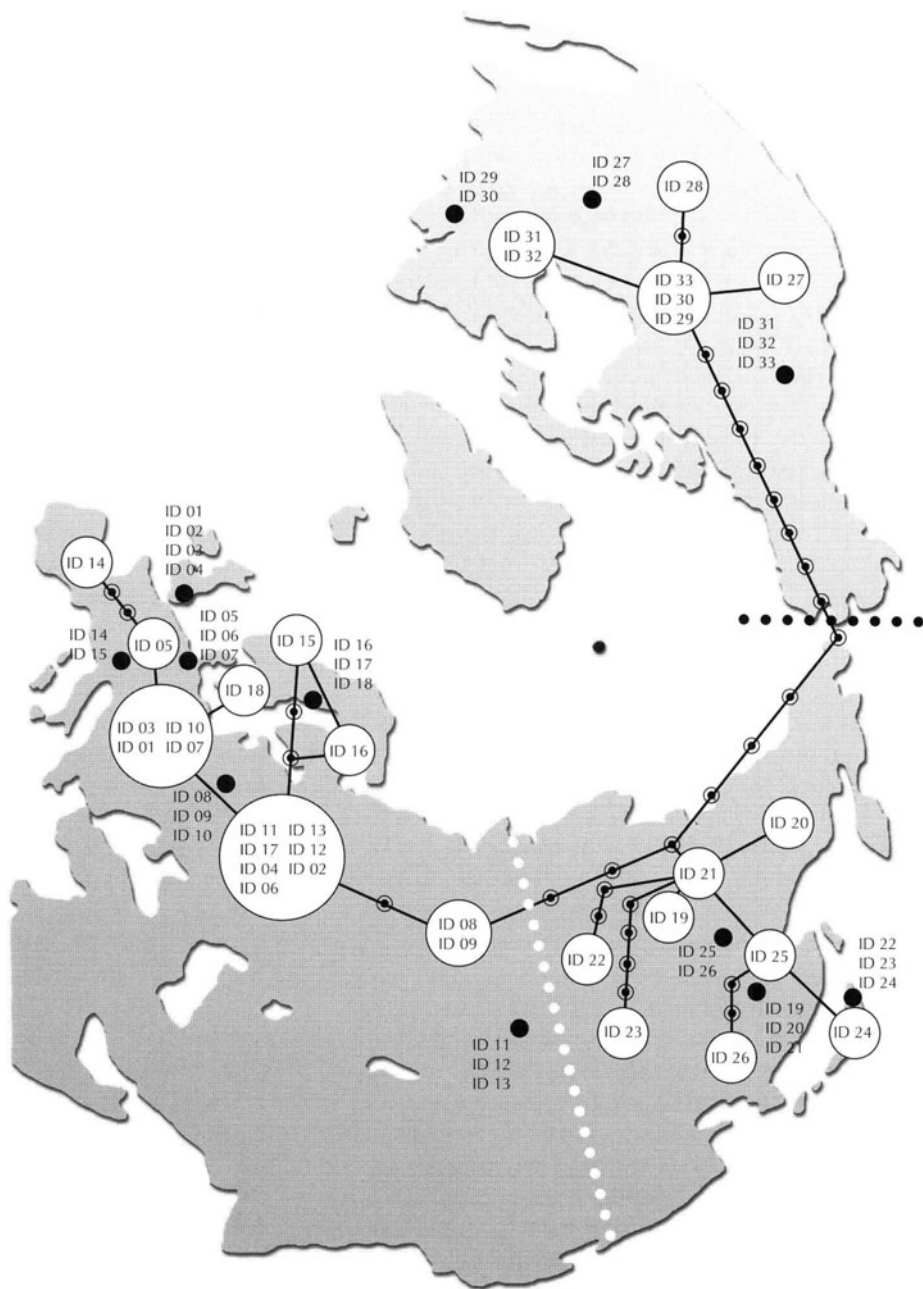
Figure 2: Parsimony network for ITS sequences of *Cordulia aenea, C. amurensis* and *C. shurtleffii*. Despite haplotype variability within the three forms, they all are clearly separated units in this parsimony network. Large circles contain ID numbers representing identical haplotypes, small circles connecting large circles represent unobserved haplotypes. The number of un-observed haplotypes is congruent to the number of steps between observed haplotypes. Black dots refer to the collecting sites (cf. Table 1); dottet lines separate the taxa.

Our conclusions are based on two main assumptions. First, we assume that the ITS tree is congruent to the *Cordulia* species tree. This assumption is indeed a bit overdrawn, as gene trees of closely related populations or species often fail to yield congruent pictures due to variable independent lineage sorting periods within the populations (for a review, see Avise 2000). Additional genetic data from independent loci is needed to sum up a "central tendency" (Avise 2000: 285) of those gene trees which will actually represent the species phylogeny.

The observed high level of variability within the genus *Cordulia* is striking. The sequences of ITS haplotypes illustrate a fairly pronounced differentiation between the three groups. These haplotypes are not easily transformed by single substitutions into each other but are clearly differentiated at various sites. It is obvious that the present sampling is far from exhaustive. This deficiency in sampling might strongly bias our analysis and prevent us from discovering the overlapping haplotypes among *C. aenea* and *C. amurensis* populations. However, the present geographic distribution of sampled specimens covers the complete Palaearctic region. Additionally, the sampling within the distributional ranges of *C. aenea* and *C. amurensis* was done randomly and was not closely clustered. If "*aenea*" haplotypes occur in "*amurensis*" phenotypes or ranges, we would expect to discover them even in our admittedly coarse sampling regime. Consequently, we are convinced that a potential sampling bias does not heavily influence our results and conclusions.

Although there is considerable variability within *Cordulia*, we did not record many heterozygotes for individual specimens, except in five possible instances in which the sequence signal was not totally unequivocal. This phenomenon is known for the nuclear ribosomal RNA gene clusters and is not yet fully understood (Hillis & Dixon 1991; see also e.g. Cruickshank 2002). In general, sequences of the ITS I non-coding region have been clear and unambiguous; hence, we think that the high variability despite the absence of heterozygotes or heterogeneity is not a PCR artefact. The NJ-trees do not resolve relationships within *C. aenea*, *C. amurensis* and *C. shurtleffii*. Apparently, there is no phylogenetic structure among haplotypes within the species.

Using parsimony networks, we found multiple haplotypes without a clear geographically structured distribution in *C. aenea* and *C. amurensis* (Fig. 2). This distribution of haplotypes for both species is most congruent to a pattern generated by rapid recolonization of habitats, probably after the glacial period. Rapid extension of distributional range is not surprising for an insect like *C. aenea*. These dragonflies are excellent fliers with potentially high individual moving distances. Therefore, we conclude that *C. aenea*, *C. amurensis* and *C. shurtleffii* represent three separate biological species (sensu Mayr 1942) with wide ranging pan-mixing populations.

Alternatively, the observed genetic differentiation between *C. aenea* and *C. amurensis* could be explained through isolation by distance which would imply a clinal differentiation between these two forms rather than a real speciation phenomenon. However, geographic distances between *C. aenea* collection localities exceed those between some collection localities of *C. aenea* and *C. amurensis*, but haplotypes are consistently assigned to either of the two genetic clusters. This pattern of geo-

graphical distribution of haplotypes is not consistent with the hypothesis of isolation by distance.

Our interpretation of the data rests on the assumption that every established biological species will acquire autapomorphic alleles even in selectively neutral loci within pan-mixing populations due to a complete elimination of gene flow and lineage sorting. This is obviously the case for the ITS I variation between *C. aenea*, *C. amurensis* and *C. shurtleffii*, as we find autapomorphic character states and exclusive genetic variability associated with phenotypes. Of course, speciation is more than the acquisition of monophyly at presumably selectively neutral genetic markers, which is achieved in every allopatric population depending on population size and time of isolation. It can be expected that selectively neutral markers travel freely within populations. We can speculate that *C. aenea* and *C. amurensis* acquired exclusive haplotypes in allopatric refugia and came into secondary contact after the retreat of the ice shield. If the species status of both forms did not reach intrinsic reproductive isolation, we would expect the free exchange of selectively neutral genetic markers across distributional ranges. At least for the ITS I marker, which is clearly selectively neutral, this is not the case. The observed ITS variation suggests a good species status of *C. shurtleffii*, *C. aenea* and *C. amurensis*.

Most significant is the final acquisition of complete reproductive isolation and ecological differentiation, which can not be analysed by the sole reliance on phylogenetic approaches. An exhaustive analysis of the differentiation status of *C. aenea* and *C. amurensis* will require an even denser sampling at the presumed East Asian contact zone. It will be interesting to see whether *C. aenea* and *C. amurensis* actually form a narrow contact zone with limited gene flow and a clear evolutionary (ecological) differentiation establishing reproductive isolation between these two species.

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