

Chromosome numbers in *Aconitum* sect. *Cammarum* (Ranunculaceae) from the Carpathians

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Abstract — Chromosome numbers for four species and two nothospecies of the sect. *Cammarum* ser. *Variegata*, ser. *Toxicum*, and nothoser. *Toxigata* in the Carpathians amount to $2n=16$, and in one case to $2n=32$. For the nothospecies: *A. ×gayeri* Starmühl. and *A. ×pawlowskii* Mitka et Starmühl. chromosome numbers are given for the first time. According to the cytological evidence a new combination *Aconitum degenii* subsp. *degenii* var. *intermedium* (Zapał.) Mitka [= *A. hebegynum* non DC.] is proposed.

Key words: *Aconitum* sect. *Cammarum*, chromosome numbers, hybridization, Linnaean taxonomy, Ranunculaceae.

INTRODUCTION

The genus *Aconitum* L. is circumscribed within three subgenera: *Aconitum*, *Lycotconum* (DC.) Peterm. and a monospecific *Gymnandrum* (Stapf.) Rapaics (LIANGQIAN and KADOTA 2001). Additionally, some authors distinguish the subgen. *Anthora* DC. (Peterm.) (= *Anisanthora* Nakai) and subgen. *Tangutica* (W.T. Wang) Kadota (2001), though their rank in the Linnean systematics of the genus is not clear. In Europe, the taxonomic treatment of subgen. *Aconitum* is clear: the section *Cammarum* (*Aconitum variegatum*-Group sensu GÖTZ 1967) includes only diploid species, and sect. *Aconitum* (*A. napellus*-group sensu SEITZ 1969) possesses tetraploid species. The species of the sect. *Aconitum* grow in open, high-mountain alpine and subalpine zones, and of the sect. *Cammarum* mainly in forest montane zones, sometimes having the status of the montane species descending to the lowlands (MITKA 2003). Opposite to Europe, the systematics of the Asian

subgen. *Aconitum* is still far from being satisfactorily understood (LUO *et al.* 2005). The reasons of taxonomic inconsistency include the high level of morphological variation due to morphological plasticity and the notorious interspecific hybridization (KADOTA 1981; OH and PARK 1998), also between species of the different ploidy levels (ZIELIŃSKI 1982a, b).

The general taxonomy of sect. *Aconitum* in the Carpathians and the chromosome numbers were given in our previous paper (ILNICKI and MITKA 2009). Now, we report the chromosome numbers for *A. sect. Cammarum* in the mountain range.

The Carpathians are the most important centre of *Aconitum* evolution in Europe (MUCHER 1993; STARMÜHLER and MITKA 2001; MITKA 2003). The geomorphology of the mountain range is linked with its geographical and geobotanical division into the Western, Eastern, and Southern Carpathians (KONDRACKI 1989; ZEMANEK 1991). Especially, the Western Carpathians are isolated from the rest of the mountain range through the low situated the Beskid Niski (Low Beskyd Mts.) and the lowest lying in the Carpathian arch the Dukla Pass - 1000 m above the sea level. The geographical isolation is accompanied by schizogenemism in various genera in the Western Carpathians (e.g. MRÁZ and SZELĄG 2004; RONIQUIER *et*

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al. 2008; TEŠITEL *et al.* 2009), including *Aconitum*. For example, in the Western Carpathians two infraspecific taxa of *Aconitum firmum* Rchb., i.e. *A. f.* subsp. *moravicum* Skalický and *A. f.* subsp. *maninense* (Skalický) Starmühl., and nothospecies *A. ×pawlowskii* (Pawl.) Mitka *et* Starmühl., are endemic to the region (STARMÜHLER and MITKA 2001; MITKA 2003).

All examined *Aconitum* subgen. *Aconitum* karyotypes, both European and Asian, are highly asymmetric and always consist of two big and six smaller chromosomes (SHANG and LI 1984; JOACHIMIAK *et al.* 1999; YANG 2001). The sect. *Cammarum* is diploid with $2n=16$ (SEITZ 1969; JOACHIMIAK *et al.* 1999). The results of studies on the sect. *Cammarum* ser. *Variegata*, i.e. *A. variegatum* subsp. *variegatum*, and two other species of the sect. *Cammarum* ser. *Toxicum*: *A. lasiocarpum* and *A. degenii*, showed high similarity of their chromosome morphologies. The only difference concerned the stability of the satellites. In *A. variegatum* three pairs of NOR chromosomes (I, III and V) had the same size of the satellites, but in the karyotype of the remaining species the chromosomes of I and III pairs showed structural heterozygosity, i.e. only in one of the homologues of the pair the satellite was clearly visible (JOACHIMIAK *et al.* 1999).

In our previous study (JOACHIMIAK *et al.* 1999) we also presented an accurate organization of the classical and C-banding karyotype of *A. lasiocarpum* as well as the estimated content and distribution of heterochromatin in *A. variegatum* subsp. *variegatum* and *A. degenii*. The karyotype of the examined specimens of *A. variegatum* was rich in heterochromatin, in contrast to *A. lasiocarpum* and *A. degenii*, which were poor in this fraction. We also stated the same structural heterozygosity of the NOR-chromosomes of I and III pairs of *A. lasiocarpum* and *A. degenii* in terms of the size of heterochromatic segments neighbouring with NOR on their shorter arms. These results didn't confirm GÖTZ'S (1967) hypothesis on the hybridogenous origin of *A. lasiocarpum*, which could presumably arise in the effect of crossing between *A. variegatum* and *A. degenii*. On the other hand, the structural heterozygosity shows evolutionary lineage of *A. lasiocarpum* and *A. degenii* (JOACHIMIAK *et al.* 1999), circumscribed in the ser. *Toxicum* (MUCHER 1993).

In the present study, we examined eight taxa of the sect. *Cammarum* from the Carpathians: *A. variegatum* subsp. *variegatum*, *A. v.* subsp. *nasutum*, *A. lasiocarpum* subsp. *lasiocarpum*, *A. l.* subsp. *kotulae*, *A. degenii* and *A. toxicum* and two hybrid, i.e. *A. ×pawlowskii* (*A. lasiocarpum*

× A. variegatum subsp. *variegatum*) and *A. ×gay-eri* (*A. lasiocarpum* *× A. degenii*). The present results could be helpful in the explaining of the taxonomic relations among taxa of the sect. *Cammarum*. The results could also contribute to better understanding of evolutionary pathways in the genus *Aconitum*.

MATERIALS AND METHODS

Aconitum specimens were collected in the field and transferred to the Botanical Garden of the Jagiellonian University, Kraków, Poland. The specimens used in the study are kept in the herbarium of the Institute of Botany of the Jagiellonian University KRA.

The method of staining of the chromosomes is described in the paper by JOACHIMIAK *et al.* (1999).

The taxonomic system follows STARMÜHLER (2001) and geographical names follow KONDRACKI (1989).

RESULTS AND DISCUSSION

Aconitum sect. *Cammarum* DC. subsect. *Cammarum* ser. *Variegata* Steinb. ex Starmühl. (2001).

Ser. *Variegata* is characterized by homozygosity of the largest pair of the chromosomes (no. 1) in the karyotype concerning the occurrence of satellites - JOACHIMIAK *et al.* (1999).

Aconitum variegatum L. (1753)

Aconitum variegatum subsp. *variegatum* - $2n=16$ (Fig. 1A)

Locality: [Poland] Tylmanowa, Gorce Mts., Western Carpathians, c. 520 m, 27.09.1999 J. Mitka (KRA).

A subspecies endemic to Europe. It belongs to the European-temperate-montane group and to the Alpic-Central European distributional type (ZAJĄC and ZAJĄC 2009). It occurs in the Alps, Pyrenees, northern Apennines, Sudetes, Eastern and Western Carpathians. In the Western Carpathians it reaches the north-eastern limit of the geographical distribution along the Wisłoka river in the Niski Beskyd (Poland). The ecological center of the species in the Western Carpathians is in the Tatra Mts., where it can be found sporadically up to 1500 m above the sea level.

Apart from the Carpathians *A. variegatum* was karyologically analyzed in Bulgaria by KOEVA-TO-

DOROVSKA (1992) and in Austria by SCHAFER and LA COUR (1934), and SEITZ (1969). In each case diploid number $2n=16$ was noted. For information on karyology of the species in Europe see SIMON *et al.* 1999. Diploid number of the chromosomes ($2n=16$) for typical representative of the species was confirmed - see Tab. 1. We found homozygosity of the satellites on the two largest chromosomes of the subspecies *A. variegatum* subsp. *variegatum* (Fig. 1A, marked with asterisk).

Aconitum variegatum subsp. *nasutum* (Fisch. ex Rchb. em. Rupr.) Götz (1967) - $2n=16$

Locality: [Romania] Cluj-Napoca, Fâget forest, Transilvania, c. 540 m, 5.08.2007 J. Mitka (KRA).

According to GÖTZ (1967) the subspecies occurs in Europe and in the Caucasus. In Europe it can be found in the Eastern Alps, Eastern Carpathians (the Rodna, Călimăn-Harghita Mts.), Apuseni Mts., Banat, Transilvania, Balkan Mountains (the Velika Plaznica, Stara Planina, Rila, Vitoša, Rodopes). Outside Europe in Asian part of Turkey (DAVIS 1965) and in the Caucasus (LUFEROV 2000). According to LUFEROV (2000) the Caucasian *A. nasutum* differs from the European morphotypes by the plagiorthropic, chain-

orientated tubers, thus it belongs to the sect. *Catenata* Steinb. ex H. Riedl (1978). The taxonomic position of *A. nasutum* from Asia Minor is also not known. According to the karyological data by SIMON *et al.* (1999) three ploidy levels are to be found in *A. nasutum*: $2n=16, 28, 32$. The diploid number of chromosomes was found in the Caucasus (TUMAJANOV and BERIDZE 1968) and Bosnia and Herzegovina (SEITZ 1969), aneuploid and tetraploid number in Turkey (BEYAZOGLU *et al.* 1994) and tetraploid number in the Alps (SEITZ *et al.* 1972) and the Big Caucasus (GAGNIDZE *et al.* 1985). The taxon needs taxonomic and nomenclatural revision. Diploid number of the chromosomes ($2n=16$) of the studied subspecies was confirmed. In our studies on *Aconitum variegatum* subsp. *nasutum*, homozygosity of the largest pair of the chromosomes in the karyotype concerning the occurrence of the satellites was noted.

Aconitum sect. *Cammarum* DC. subsect. *Cammarum* ser. *Toxicum* (Rchb.) Mucher (1993).

Series *Toxicum* is morphologically characterized by glandular hairs in the inflorescence, and karyologically by heterozygosity of satellites on the largest pair of the chromosomes (no. 1) - JOACHIMIAK *et al.* (1999).

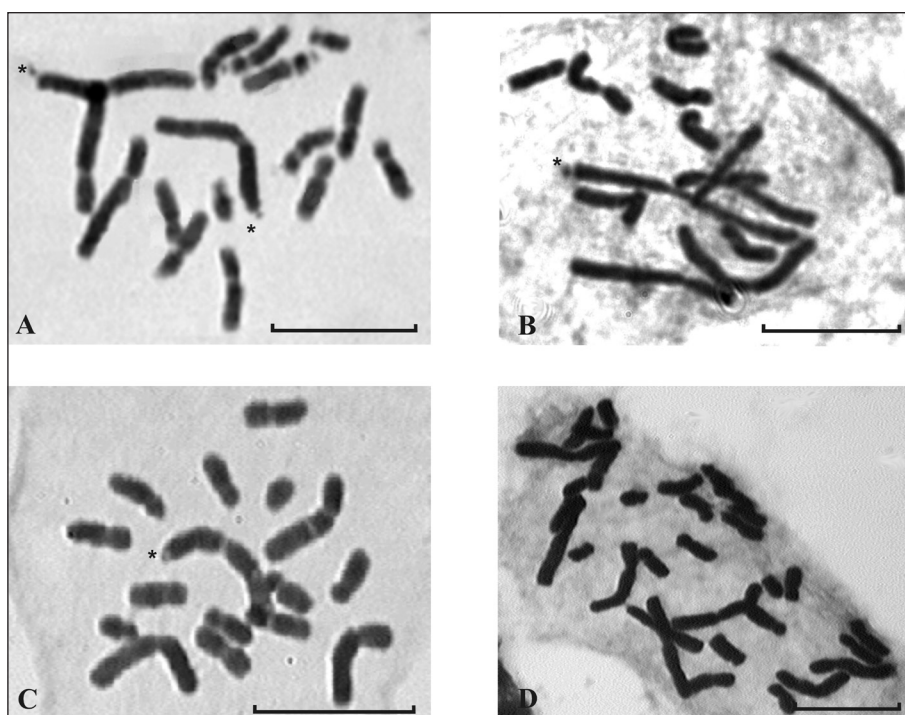


Fig. 1 — Metaphase plates of the studied taxa of *Aconitum* sect. *Cammarum* from the Carpathians (A – *A. variegatum* ($2n=16$), B – *A. lasiocarpum* subsp. *kotulae* ($2n=16$), C – *A. degenii* ($2n=16$), D – *A. degenii* ($2n=32$). * satellite, scale bar = 10 μm .

Aconitum degenii Gáyer = *A. paniculatum* Lam. *nom. inval.*

A. degenii subsp. *degenii* - $2n=16, 32$ (Fig. 1C, D)

Locality: [Ukraine] Polonina Pożyżewska, Chornogora Mts., Eastern Carpathians, c. 1300 m, 1.08.1999 J. Mitka (KRA) (Fig. 1 C); [Romania] Mt. Pietrosul, Rodna Mts., 1640 m, 17.08.1998 J. Mitka, P. Bochenek (Fig. 1 D) (KRA).

A. degenii subsp. *degenii* is a Carpathian subspecies endemic to E Carpathians, S Carpathians, and the Apuseni Mts. A distinct morphotype with capitate spur of the nectaries fo. *craciunelense* Gáyer (1906) occurs in E Carpathians in the Rodna, Chyvchyn and Chornogora Mts. *Aconitum degenii* in the Alp and Balkan Mountains is circumscribed within the subspecies *A. d.* subsp. *paniculatum* (Arcang.) Mucher and in the Alps as *A. d.* subsp. *valesiacum* (Gáyer) Mucher (1993). Diploid number of the chromosomes ($2n=16$) for typical representative of the species was confirmed - see Tab. 1. We also noted a tetraploid form of the subspecies *A. degenii* subsp. *degenii* $2n=32$ (Fig. 1D) and the heterozygosity of the satellites on the largest pair of the chromosomes (Fig. 1C, marked with asterisk).

Aconitum degenii Gáyer subsp. *degenii* var. *intermedium* (Zapał.) Mitka *comb. nov.* - $2n=16=A. bebegynum$ non DC., *auct. fl. Carp. Orient.* (MITKA 2000, 2003).

Locality: [Ukraine] Zaroślak on the Prut river and Polonina Pożyżewska, Chornogora Mts., Eastern Carpathians, c. 1275-1410 m, 1.08.1997 J. Mitka (KRA).

An Eastern-Carpathian variety endemic to E Carpathians: the Bieszczady, Svidovec-Negrovec, Chornogora, Bucovina, Gorgans and Călimăh-Harghita Mts. (MITKA 2000; SZAJNA and MITKA 2010). ZAPAŁOWICZ (1908) described a variety within *A. paniculatum* (= *A. degenii*) "*pedicelli sub flore sparse pilosi, cassis angusta*". The taxon, typified in MITKA (2003), is characterized by pubescent helmet, carpels glabrous and pedicels below bracteoles glabrous (NOVIKOFF and MITKA 2011) or carpels pubescent and helmet and pedicels below bracteoles glabrous. Careful phenetic study (SZAJNA and MITKA 2010) revealed that *A. intermedium* occupies intermediate position between the *A. degenii* and *A. variegatum* along the Detrended Correspondence Axis 1, and an isolated position along the DCA Axis 3. Thus, *A. intermedium* fits the morphological hybrid characteristics, but cytogenetic line of evidence places it into the ser. *Toxicum*. Diploid number of the chromosomes

($2n=16$) for the studied variety of *A. degenii* was confirmed - see Tab. 1. Our cytogenetic studies showed that *A. intermedium* has structural heterozygosity in two NOR-chromosome pairs I and III of the karyotype, typical of the ser. *Toxicum*.

Aconitum lasiocarpum (Rchb.) Gáyer (1911)

Aconitum lasiocarpum subsp. *lasiocarpum* - $2n=16$

Locality: [Poland] Buk on the Solinka river, Western Bieszczady Mts., Eastern Carpathians, c. 490 m, 4.09.1996 J. Mitka (KRA).

An Eastern Carpathian subspecies endemic to the Bieszczady, Vihorlat, Chornogora, Gorgans and Chywchyn Mts. (MITKA 2000). The southern limit of its geographic distribution is in the Bistricei Mts. on Mt. Ceahlău. In the paper by JOACHIMIĄK *et al.* (1999) heterozygosity of the I, III and V pair of the chromosomes concerning the occurrence of the satellites was noted. In our paper diploid number ($2n=16$) for the typical representative of the species was confirmed - see Tab. 1.

Aconitum lasiocarpum subsp. *kotulae* (Pawł.) Starmühl. *et* Mitka in MITKA and STARMÜHL. (2000) - $2n=16$ (Fig. 1B).

Locality: [Poland] Mt. Cergowa Góra, Niski Beskyd, Western Carpathians, c. 700 m, 8.09.1998 J. Mitka (KRA).

A Western/Eastern Carpathian subendemic: W Carpathians - the Doły Jasielsko-Sanockie Basin, the Niski Beskyd, Tatra Mts.; E Carpathians - the Bieszczady Mts., Góry Sanocko-Turczańskie, Vihorlat, Svidovec-Negrovec, Chornohora, Maramures, Rodna, Gorgans Mts. (MITKA 2000); on Podolia in the Ukraine (MITKA and STARMÜHLER 2000). *Aconitum lasiocarpum* subsp. *kotulae* is characterised by glandular pubescent helmets and glabrous pedicels (Podolia and E Carpathians) or curved and crisped eglandular pubescent pedicels (NOVIKOFF and MITKA 2011) (the Tatra Mts.). The typical subspecies has exclusively glandular pubescent pedicels. Maybe, a diploid form of this subspecies was found by Váchova in VÁCHOVA and MÁJOVSKÝ (1977) - see a comment below Table 1. In the present paper, heterozygosity of the satellites on the largest pair of the chromosomes was noted for *Aconitum lasiocarpum* subsp. *kotulae* (Fig. 1B, marked with asterisk).

Aconitum × *gayeri* Starmühl. (1996) - $2n=16$. (*Aconitum degenii* × *A. lasiocarpum*)

Locality: [Poland] Procisne, San river, Western Bieszczady Mts., Eastern Carpathians, c. 515 m, 6.09.1995 J. Mitka (KRA).

A nothospecies endemic to the Eastern Carpathians: the Bieszczady Mts., Mt. Vihorlat, Svidovec-Negrovec, Chornogora, Gorgany, Chywyhyn, Maramures, and Rodna Mts. (MITKA 2000). The chromosome number for this taxon is given for the first time.

Aconitum toxicum Rchb.

Aconitum toxicum subsp. *toxicum* - $2n=16$.

Locality: [Romania] Mt. Ceahlău, Bistricea Mts., Eastern Carpathians, c. 1400 m, 8.08.2007 J. Mitka (KRA).

A subspecies endemic to the Eastern/Southern Carpathians (including the Apuseni Mts.) and the Balkans (GÖTZ 1967; MUCHER 1993). In E Carpathians in the Bistricea and Călimăn-Harghita Mts.; in S Carpathians in the Bucegi, Piatra Craiului, Făgărăș and Retezat Mts. (GRINȚESCU 1953; МИНОК *et al.* 2005); in the Balkans in Bos-

nia and Herzegovina (the Sarajevo district) and in Montenegro (the Durmitor Mts., see GÖTZ 1967). MUCHER (1993) recognized two other subspecies from S Carpathians: *A. t.* subsp. *bucegiense* (Nyár.) Mucher and *A. t.* subsp. *crispulum* (Nyár.) Mucher, both endemic to S Carpathians, and two nothospecies: *A. ×dragulescuanum* Mucher (*A. degenii* × *A. toxicum*), endemic to E/S Carpathians (including the Apuseni Mts.), and *A. ×bartokianum* Starmühler (2000) (*A. toxicum* × *A. variegatum*) reported from E and S Carpathians, the Apuseni Mts., and Bosnia and Herzegovina. Diploids of this taxon were found by SEITZ (1969) in Bosnia and Herzegovina (Trebović) and in the Retezat Mts. (S Carpathians) - Tab.1. In this paper we report diploid number $2n=16$ for the material collected in E Carpathians (Mt. Ceahlău) and heterozygosity of the satellites on the largest pair of the chromosomes.

TABLE 1 — Published data on the chromosome numbers of *Aconitum* sect. *Cammarum* from the Carpathians.

Taxa	2n	Geographic origin	Reference
Ser. <i>Toxicum</i>			
<i>A. degenii</i> Gáyer subsp. <i>degenii</i>	16	Bieszczady Mts. (E Carpathians), Terebowiec valley	Frey <i>et al.</i> (1977)
<i>A. degenii</i> var. <i>intermedium</i> (Zapał.) Mitka [= <i>A. ×bebegynum non DC</i>]	16	Chornogora Mts. (E Carpathians), Zaroślak in Prut river valley and Polonina Pożyżewska	Joachimik <i>et al.</i> (1999)
<i>A. lasiocarpum</i> (Rchb.) Gáyer subsp. <i>lasiocarpum</i>	16	Bieszczady Mts. (E Carpathians), Buk on the Solinka river	Joachimik <i>et al.</i> (1999)
<i>A. lasiocarpum</i> subsp. <i>kotulae</i> (Pawł.) Starmühl. & Mitka	16*	Niski Beskyd Mts. (W Carpathians), Čereniny	Váchová in Váchová and Májovský (1977)
	16	Niski Beskyd Mts. (W Carpathians), Mt. Cergowa	Joachimik <i>et al.</i> (1999)
	16*	Góry Sanocko-Turczańskie Mts. (E Carpathians), Mt. Sobień	Kucharska in Skalińska <i>et al.</i> (1976)
<i>A. toxicum</i> Rchb. subsp. <i>toxicum</i>	16	Retezat Mts., Gura Zlata (E Carpathians)	Seitz (1969)
Ser. <i>Variiegata</i>			
<i>A. variegatum</i> L. subsp. <i>variegatum</i>	16	Tatra Mts. (W Carpathians)	Leszczak in Skalińska (1950)
	16	Tatra Mts. (W Carpathians)	Leszczak (1950)
	16	Tatra Mts. (W Carpathians)	Szweykowski and Mendelak (1977)
	16, 17	Tatra Mts. (W Carpathians)	Zieliński (1982b)
	16	Mt. Babia Góra (W Carpathians)	Migra (1982)
	16	Mt. Veľká Fatra (W Carpathians), Eubochnianska valley	Hindáková in Májovský <i>et al.</i> (1987)
	16	Tatra Mts. (W Carpathians), Kościeliska valley	Joachimik <i>et al.</i> (1999)
	16	Slovenský kras (W Carpathians), Mt. Jelení vrch	Uhríková in Májovský <i>et al.</i> (2000)

* According to authors *A. degenii*.

Aconitum sect. *Cammarum* DC. subsect. *Cammarum* nser. *Toxigata* Starmühl. (2001).

Aconitum ×*pawlowskii* Mitka et Starmühl. (2000) - $2n=16$ (*Aconitum variegatum* × *A. lasiocarpum*)

Locality: [Poland] Targowiska near Krosno, Niski Beskyd, Western Carpathians, 290 m, 9.09.1998 J. Mitka (KRA).

A nothospecies subendemic to W Carpathians: the Doły Jasielsko-Sanockie Basin, the Niski Beskyd, Gorce, Pieniny, Tatra Mts and forelands; the Nízke Tatry, Slovenské rudohorie, Muránska planina, and in E Carpathians in the Góry Sanocko-Turczańskie Mts. It occurs in contact zone of *Aconitum variegatum* and *A. lasiocarpum*, where form taxonomically mixed populations. It is a morphotype with glandular indumentum (as *A. lasiocarpum*) and carpels pilose on the suture (as *A. variegatum*). A cytogenetic research with C-banding method showed the hybridogenous character of *A. ×pawlowskii* (ILNICKI, unpubl.). The chromosome number for this taxon is given for the first time.

Acknowledgements — The work was supported by a MNiSZW grant No. 8144/B/P01/2011/40. Authors thank prof. Andrzej Joachimiak and anonymous reviewers for valuable comments on the manuscript.

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