

## ***Chara inconnexa* Allen (Streptophyta: Charales) and taxonomic ambiguities associated with subgymnophyllous species close to *C. contraria* A. Braun ex Kütz. s.str.**

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**Résumé** – *Chara inconnexa* Allen a été trouvé dans le sud de la Sibérie orientale. Les spécimens sont décrits en détail. C'est une espèce très rare avec une large distribution et elle est principalement recensée dans les zones tempérées et subtropicales de l'hémisphère Nord. Cette espèce d'eau douce est trouvée dans différents types de plans d'eau. Les causes de la rareté de cette espèce et les aspects taxonomiques sont discutés. Considérant que l'holotype ne pouvait pas être identifié aux fins d'assurer le lien entre le nom de ce taxon et la description, son épitype devrait être conservé. Les problèmes taxonomiques des espèces subgymnophyllous et des formes proches de *C. contraria* A. Braun ex Kütz. s.str. sont décrits. La réévaluation de la valeur taxonomique des caractères de Rameau et l'application de critères supplémentaires dans l'approche polyphasique sont nécessaires. L'ensemble de ces caractères permettra de reconnaître des espèces subgymnophyllous comme une espèce distincte ou formes de *C. contraria* ou confirmer l'hypothèse que ce sont simplement un stade de l'ontogenèse ou morphogenèse anormale de *C. contraria*.

***Chara inconnexa* / charophyte / Sibérie / Russie / espèce subgymnophylle / taxonomie**

**Abstract** – *Chara inconnexa* Allen has been found in the south of eastern Siberia. The specimens are described in detail. *Chara inconnexa* is a very rare species with a wide distribution range, but mostly inhabits the temperate and subtropical zones of the Northern Hemisphere. This freshwater species has been found in different types of water bodies. The possible causes of rare reports of *C. inconnexa* are presented. The taxonomical aspects are discussed. Whereas the holotype could not be critically identified for purposes of the precise application of the name to this taxon, its epitype should be retained. The taxonomic problems with subgymnophyllous species and forms close to *C. contraria* A. Braun ex Kütz. s.str. are outlined. The reassessment of the taxonomic value of branchlet characters and additional criteria application within a polyphasic approach are strongly required, as this will allow the recognition of subgymnophyllous species as a distinct species or forms of *C. contraria* or else will confirm the assumption that these are merely stages of ontogenesis or abnormal morphogenesis of *C. contraria*.

***Chara inconnexa* / charophyte / Siberia / Russia / subgymnophyllous species / taxonomy**

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## INTRODUCTION

Eighty-one extant macrospecies and nearly 440 microspecies of charophytes are known (Wood & Imahori, 1965; Khan & Sarma, 1984). In regional checklists, the microspecies concept is useful and widely accepted. However, from a global perspective these regional microspecies seem more like “constellations” or “clouds” of intergrading taxa around widely distributed or cosmopolitan species (Wood & Imahori, 1965). The clarification of charophyte species concept has been attempted with different approaches. The application of biological species concept has been tested with breeding experiments for species of *Chara* (Proctor, 1971; Proctor *et al.*, 1971; Proctor & Wiman, 1971; Grant & Proctor, 1972). These results as well as the combined study of oospore ultrastructure and molecular polymorphism for *Nitella* species (Sakayama *et al.*, 2002, 2004, 2005; Sakayama, 2008), and morphological and molecular polymorphism of *Tolypella* species (Pérez *et al.*, 2014) rather pointed towards suitability of microspecies concept in many cases. The morphology of the thallus and oospores and molecular polymorphism allowed distinguishing of semicryptic *Chara leptospora* Sakayama *et al.*, from *C. globularis* Thuill. (Sakayama *et al.*, 2009), as well as *C. globata* Migula from other members of *Hartmania* section (Romanov *et al.*, 2015). In contrast, the morphology of thallus and oospores, physiology and genetical polymorphism studies failed to exactly differentiate several species for *Hartmania* section of *Chara* (Boegle *et al.*, 2010 a, b; Urbaniak, 2010; Urbaniak & Combik, 2013; Schneider *et al.*, 2015). Many monoecious species with diplostephanous stipulodes, diplostichous tylacanthous or nearly isostichous stem cortex, phloeopodous branchlets and fructified corticated segments of the branchlet *i.e.* close to *C. contraria* A. Braun *ex* Kütz. *s.str.*, have variable incomplete cortication of branchlet segments or few corticated branchlet segments. These species are comparatively rare and sometimes hardly differentiable. The plant findings in eastern Siberia, which could be identified as *C. inconnexa* Allen, raise the question of taxonomic validity of these forms described as a species. The aims of this study were to describe the morphological characteristics of plants referable to *C. inconnexa* and to discuss its validity as a separate species based on material from eastern Siberia.

## MATERIALS AND METHODS

### Preparations of specimens

The specimens were collected in July and August 2006, 2008 and 2011 in eastern Siberia, Russia (Fig. 1). Specimens were treated with a 2% acetic acid solution, rinsed in distilled water, treated with a 2% potassium hydroxide solution and finally again rinsed in distilled water before examination. The photos were taken with a Carl Zeiss Stereo Discovery V12 stereomicroscope equipped with an AxioCam MRs-5 digital camera. The oospores were treated with acetic acid to remove any lime-shell, washed with distilled water and cleaned from spiral cells by adding 10% Triton X100, then stored at 60°C for at least 10 hours. Finally, they were washed with distilled water and sonicated to completely remove spiral cells. The cleaned oospores were stored in 95% alcohol. They were coated with zinc and studied using

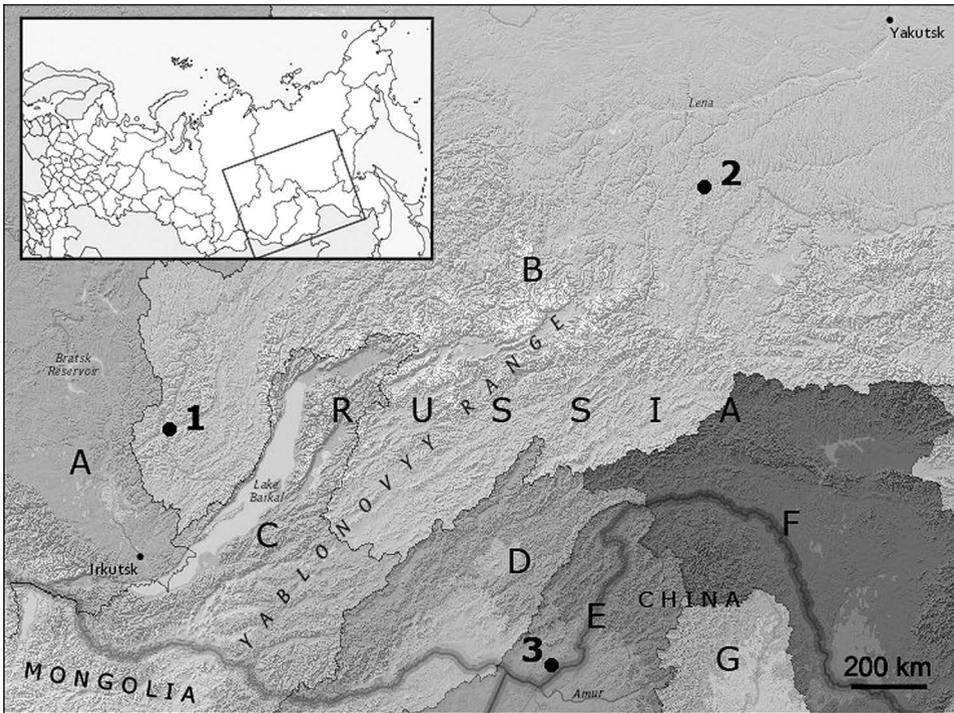


Fig. 1. Localities of *Chara inconnexa* in northern Asia within freshwater ecoregions (Abell *et al.*, 2008): 1. Tutura river, 2. Amga river, 3. Krasnokamensk reservoir, A-G ecoregions: A. Yenisei, B. Lena, C. Lake Baikal, D. Shilka, E. Argun, F. Middle Amur, G. Songhua Jiang. Map source: <http://www.feow.org>, copyright 2008 by The Nature Conservancy and World Wildlife Fund, Inc.

a ZEISS EVO 40 scanning electron microscope. The terms used for describing the oospore surface followed those of Urbaniak (2011). Herbarium specimens were deposited in NS, SASY, and in the Institute of Natural Resources, Ecology and Cryology of SB RAS (Chita).

### Description of localities

1) The Tutura River is one of the main tributaries of the upper Lena River, its length is 222 km. The mean duration of the open-water period is 180 days in the lower reach. The mean monthly discharge of water was  $35\text{--}57\text{ m}^3\text{ s}^{-1}$  from June to August 1976-1980; the mean temperature of water varied from  $12.6\text{--}16.5^\circ\text{C}$  from June to August 1950-1980 (Aknesenko & Vitkovskaya, 1986). Frequent rain floods intermitted with short-term low waterlevel periods are characteristic of its summer water regime (Makarov & Opekunova, 2010). The charophytes were collected within the near-mouth reach.

2) The Amga River is the largest tributary of the Aldan River, its length is 1360 km. The Amga channel is a chain of deep, long pools alternating with shallow riffles. This river is one of the most slow-flowing amongst the large rivers of Yakutia; its water velocity varies from  $0.3\text{--}0.6\text{ m s}^{-1}$  during the summer low-water period.

The mean duration of the open-water period is 149 days. The river bed consists of pebbles; numerous small riffles are present in upper section. The average salinity was 0.24‰, total hardness was  $2.87 \text{ mg-equiv}\cdot\text{L}^{-1}$ , pH 7.58;  $\text{HCO}_3^-$ ,  $\text{Mg}^{2+}$  and  $\text{Ca}^{2+}$  179, 23 and  $20 \text{ mg}\cdot\text{L}^{-1}$ , respectively, in the water of the upper stretch in July 2006 (Gabyshev & Gabysheva, 2011). The water was oversaturated with oxygen (110%) and had very low concentrations of organic components, inorganic nitrogen and phosphorus. Submersed aquatic angiosperms (mostly *Potamogeton* spp.) grew abundantly along the river banks. Charophytes have been found in the upper reach at a steep slope at a depth of 1.2 m, where they form a dense community that looks like a “rough wall”.

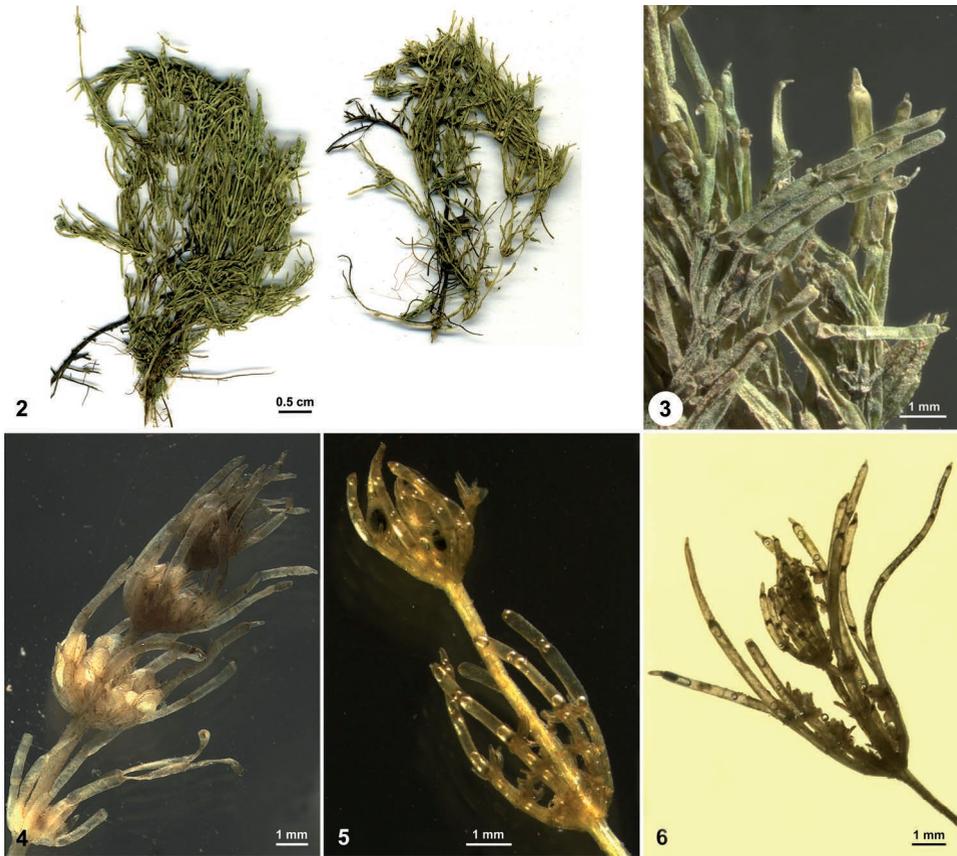
3) The Krasnokamensk reservoir was created in 1974 as a water source for the uranium Mining and Chemical Combine in the steppe zone within the Upper Amur drainage basin. This eutrophic pumped storage reservoir is filled from the Argun River. Its area is  $2.2 \text{ km}^2$ , its volume is  $15.8 \cdot 10^6 \text{ m}^3$ , its maximal depth is 16 m, the transparency of the water is 0.4-3.0 m and its salinity is 0.25-0.75‰ (Gorlacheva & Afonin, 2005). The bottom of the reservoir is formed of silt sediments. The community of charophytes was found at a depth of 2.5 m.

## RESULTS

### *Chara inconnexa* Allen 1882

#### Description

The strongly incrustated fragile plants with scarce or abundant rhizoids were 4-11 cm in height (Fig. 2). The collected specimens resembled moss turf in their general appearance due to stems, which emerged from the common tangle of rhizoids or basal part of stems were sometimes clustered and matted. Ecorticate lower stem internodes were present in specimens from the Amga River. They were colourless and transparent at the base and sometimes emerged from basal nodal bulbils. The stem diameter was  $360\text{-}570 \mu\text{m}$ . The stem cortex was diplostichous, tylacanthous or rarely isostichous (Fig. 8). The secondary tubes were joining obliquely. Sometimes, patches of an irregular stem cortex were formed. The spine cells were solitary, papillose or slightly elongated. The short ellipsoid or slightly elongated stipulodes were in double rows, uppers are usually longer than lowers. The branchlets were 5.2-14.5 mm in length and usually arcuately incurved in corticated parts (Figs 4, 9). The total number of branchlet segment was 5 or 6. The ecorticate segment was the main part of the branchlet (Figs 3-8). The corticated segments were short and abbreviated. Sometimes, corticated segments, bract-cells, bracteoles and gametangia were conglomerated, so the lowest parts of the branchlets were distinct in habit of thalli (Fig. 4). The branchlet cortex was diplostichous and complete. The relative length of the ecorticate segment was apparently less in the upper immature branchlets compared to mature branchlets with the same number of corticated segments, which could be explained by a larger elongation of the ecorticate segment during the branchlet growth in comparison with the corticated segments. The numbers of cells in the ecorticate segment are inversely correlated with the number of corticated segments (Table 1). The cell length gradually decreased towards the end of each ecorticate segment (Figs 3-6, 8). The last cell of each ecorticate segment was short



Figs 2-6. *Chara inconnexa*, LM: **2**. Overview of herbarium specimens, **3-6**. Upper parts of shoots with characteristic branchlets consisting of a few short abbreviated corticated differentiated segments and long ecorticate undifferentiated segments: **3**. Dry specimen, **4-6**. Specimens treated with acetic acid and subsequently potassium hydroxide.

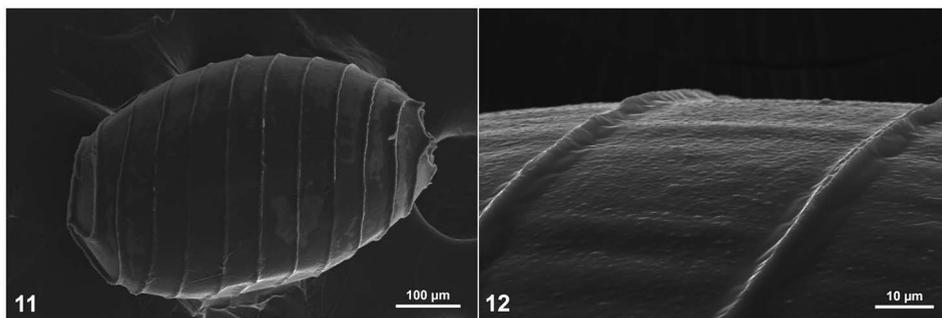
and conical, but not mucronate. The bract cells were unilateral and the posteriors were rudimental (Figs 9, 10).

The gametangia were present in almost all well-developed whorls including all upper ones; they were solitary, conjoined and located between or adjacent to corticated segments or between ecorticate segments in a single case only (Figs 4-7, 9, 10). The oogonia were rarely geminate. Only a few ripe or presumably incompletely ripe oospores were available in the studied collections. The oospore surface ornamentation was pustular (Figs 11, 12); numerous small pustular projections of variable size (0.5-1.0  $\mu\text{m}$  in diameter, sometimes with pores at the tips) were arranged closely together in specimens from the reservoir. The studied specimens from different water bodies were not identical (Table 1).

**Localities** (Fig. 1): Russia, Eastern Siberia: 1. Upper Lena drainage basin, Irkutsk Oblast', Zhigalovsky District, in the vicinity of the village of Tutura, 54°47'08"N 105°14'02"E, Tutura River, near-mouth reach, 24-vii-2008, unknown collector, NS; 2. Upper Lena drainage basin, Sakha Republic (Yakutia Republic),



Figs 7-10. *Chara inconnexa*, LM: **7**, **8**. Whorls of branchlets, **9**. Abbreviated corticated segments of branchlets, **10**. Oogonium, bract cells and bracteoles on node within the ecorticate part of the branchlet (arrowhead).



Figs 11-12. SEM-images of oospores of *Chara inconnexa*: **11**. Overview of the oospore with fields of densely perforate ornamentation surrounded by smooth areas, **12**. Part of the oospore with pustular ornamentation.

Amga Ridge, 59°19'59"N 123°31'47"E, Amga River, upper reach, community of *C. inconnexa* and *C. globularis* Thuill., 19-viii-2011, N.K. Sosina, SASY; 3. Upper Amur drainage basin, Zabaikalskii Krai, Krasnokamensky District, 49°59'09"N 118°12'27"E, Krasnokamensk reservoir, 17-vii-2006, B.B. Bazarova, NS, herbarium of Institute of Natural Resources, Ecology and Cryology of SB RAS, NS.

Table 1. The morphological characters of studied specimens of *Chara inconnexa*

<i>Characteristic</i>	<i>Tutura River</i>	<i>Amga River</i>	<i>Krasnokamensk reservoir</i>
Stem cortex	tylacanthous or rarely isostichous	tylacanthous	tylacanthous
Relative lengths of stem internodes in comparison with branchlet	1.5 to 2.5-fold shorter	nearly equal or slightly shorter	~ 1 to 1.3-fold or several times longer
Relative length of corticated part of branchlet	0.16-0.31	0.06-0.38	0.16-0.31
Number of corticated segments and cells in ecorticate segments in branchlet	6, rarely 5	5-6	5-6
Number of corticated segments in branchlet	2, rarely 1	1-3, mostly 2, rarely 1;	1-4; mostly 2 or 3
Number of cells in ecorticate segment	4, very rarely 3	3-4	2-4
Relative length of first cell in ecorticate segment in comparison with ultimate corticated segment	1.8 to 2.6 mostly 2.0 to 2.2-fold longer	1.2 to 5.7-fold longer	1.7 to 5.5-fold longer, mean 2.9 (n = 22)
Relative length of anterior bract cells and bracteoles in comparison with oogonium	0.5-0.7-1	0.6-0.9-1.0	1.0-1.1
Relative length of anterior bract cells in comparison with bracteoles	slightly shorter	nearly equal	slightly or to 1.5-fold shorter, rare slightly longer
Relative length of oogonium in comparison with adjacent corticated segment	frequently equal	shorter	shorter
Oogonium dimensions without coronula, length × width, μm	526-927 × 415-618	515-880 × 355-560	744 × 551
Oogonium coronula dimensions, length × width, μm	97-137 × 246-286	86-137 × 212-320	–
Oospores dimension, length × width, μm	–	486-652 × 320-412	494–679 × 278–359
Colour of oospore membrane in transmitted light, degree of maturity	colourless, unripe only	black, ripe	brown, incompletely ripe (?)
Antheridium diameter, μm	303-418	195-366	–

Note: n – number of measurements.

## DISCUSSION

## Taxonomy

The synonyms and names of records referable to *Chara inconnexa* are listed in Table 2. *Chara inconnexa* from Storm Lake, Iowa, in North America was originally described by Allen (Allen, 1888; Robinson, 1906; Prescott, 1931). A reinvestigation of the holotype deposited in NY by Wood & Imahori (1964, 1965)

Table 2. Synonyms and names of records referable or possibly referable\* to *Chara inconnexa*

Year of publication	Names	References
1882	<i>Chara inconnexa</i> Allen	Allen: 40, Pl. 17
1906	<i>C. inconnexa</i>	Robinson: 264
1906	<i>C. hypnoides</i> C.B.Rob.	Robinson: 263
1914	<i>C. contraria</i> A. Braun ssp. <i>hippelliana</i> Vilh.	Vilhelm: 102, 103
1923	<i>C. hippelliana</i> (Vilh.) Vilh.	Vilhelm: 150
1931	<i>C. inconnexa</i>	Prescott: 141, Pl. 37 (after Allen)
1947	<i>C. arrudensis</i> Mendes	Mendes: 285, figs 1-5
1948	<i>C. arrudensis</i>	Mendes: 433
1952	<i>C. inconnexa</i>	Wood: 326
1952	<i>C. hypnoides</i>	Wood: 326
1957	<i>C. contraria</i> (incl. <i>C. arrudensis</i> )	Corillion: 179
1959	<i>C. inconnexa</i>	Wood: 188, 191, 192
1959	<i>C. hippelliana</i>	Wood: 191
1959	<i>C. hypnoides</i>	Wood: 187, 191, 192
1962	<i>C. vulgaris</i> L. var. <i>inconnexa</i> (Allen) R.D. Wood f. <i>inconnexa</i>	Wood: 8
1962	<i>C. vulgaris</i> var. <i>inconnexa</i> f. <i>hippelliana</i> (Vilh.) R.D. Wood	Wood: 8
1962	<i>C. vulgaris</i> var. <i>inconnexa</i> f. <i>arrudensis</i> (Mendes) R.D. Wood	Wood: 8
1964	<i>C. vulgaris</i> var. <i>inconnexa</i> (Allen) R.D. Wood, em. f. <i>inconnexa</i>	Wood & Imahori: Icon 20
1964	<i>C. vulgaris</i> var. <i>inconnexa</i> f. <i>inconnexa</i> (= <i>C. hypnoides</i> )	Wood & Imahori: Icon 21
1964	<i>C. vulgaris</i> var. <i>inconnexa</i> f. <i>hippelliana</i>	Wood & Imahori: Icon 22
1964	<i>C. vulgaris</i> var. <i>inconnexa</i>	Wood: 37
1965	<i>C. vulgaris</i> var. <i>inconnexa</i> f. <i>inconnexa</i>	Wood & Imahori: 118, 119
1965	<i>C. vulgaris</i> var. <i>inconnexa</i> f. <i>hippelliana</i>	Wood & Imahori: 119, 120
1965	<i>C. vulgaris</i> var. <i>inconnexa</i> f. <i>arrudensis</i>	Wood & Imahori: 121
1966	<i>C. contraria</i> (= <i>C. inconnexa</i> -like)*	Tindall: 228
1968	<i>C. inconnexa</i> intermediate between <i>C. vulgaris</i> var. <i>inconnexa</i> f. <i>inconnexa</i> and f. <i>arrudensis</i>	Corillion: 105, 106

<i>Year of publication</i>	<i>Names</i>	<i>References</i>
1971	<i>C. vulgaris</i> var. <i>inconnexa</i> f. <i>inconnexa</i> R.D. Wood (incl. <i>C. contraria</i> ssp. <i>hippelliana</i> )	Corillion & Guerlesquin: 183, 184
1971	<i>C. vulgaris</i> var. <i>arrudensis</i> [ <i>arrudensis</i> ] (Mendes) R.D. Wood	Rich <i>et al.</i> : 11
1972	<i>C. vulgaris</i> var. <i>inconnexa</i> f. <i>inconnexa</i>	Corillion & Guerlesquin: 24-26, Pl. 1: j
1973	<i>C. inconnexa</i>	Corillion: 69, 73, 75
1974	<i>C. vulgaris</i> var. <i>inconnexa</i>	Frame: 310
1977	<i>C. inconnexa</i>	Corillion: 52
1977	<i>C. inconnexa</i>	Ebinger & Vogel: 99
1978	<i>C. inconnexa</i>	Corillion: 29
1982	<i>C. vulgaris</i> var. <i>inconnexa</i>	Altinayar & Onursal: 126
1986	<i>C. vulgaris</i> var. <i>inconnexa</i>	Compère: 47
1986	<i>C. vulgaris</i> var. <i>inconnexa</i> f. <i>hippelliana</i>	Ray & Chatterjee: 663
1988	<i>C. vulgaris</i> var. <i>inconnexa</i> f. <i>hippelliana</i>	Pal & Chatterjee: 2
1989	<i>C. vulgaris</i> var. <i>inconnexa</i> without determination to the form sensu R.D. Wood due to variability of characters within studied specimens	Mann: 103
1990	<i>C. arrudensis</i>	Su <i>et al.</i> : 340, Fig. 6: 6-9
1994	<i>C. inconnexa</i> (= <i>C. arrudensis</i> , <i>C. pistianensis</i> Vill.)	Han & Li: 205, 206, Fig. 156
1994	<i>C. contraria</i> with combination of unusual characters within robust thalli, these specimens differ from <i>C. inconnexa</i> by peculiar prominent apical projections on the oospore	Mann: 418, Fig. 2
2000	<i>C. inconnexa</i>	Ling <i>et al.</i> : 86
2005	<i>C. vulgaris</i> var. <i>inconnexa</i>	Aysel: 36
2007	<i>C. vulgaris</i> var. <i>inconnexa</i> (= <i>C. arrudensis</i> )	Cirujano <i>et al.</i> : 91
2008	<i>C. vulgaris</i> var. <i>inconnexa</i> (= <i>C. arrudensis</i> )	Cirujano <i>et al.</i> : 58, Fig. 41 (after Wood & Imahori)
2008	<i>C. inconnexa</i> (= <i>C. arrudensis</i> )	Zhang <i>et al.</i> : 594, 595, figs 1, 2
2009	<i>C. hippelliana</i>	Caisova & Gabka: 5
2009	<i>C. vulgaris</i> var. <i>inconnexa</i>	Anonymous: 345
2010	<i>C. vulgaris</i> (= <i>C. inconnexa</i> , <i>C. hypnoides</i> )	Scribailo & Alix: 43
2010	<i>C. inconnexa</i>	Han <i>et al.</i> : 601
2010a	<i>C. inconnexa</i>	Zhang <i>et al.</i> : 456
2010b	<i>C. inconnexa</i>	Zhang <i>et al.</i> : 390
2012	<i>C. inconnexa</i>	Shi <i>et al.</i> : 4176
2014	<i>C. inconnexa</i>	Barinova <i>et al.</i> : 39
2014	<i>C. inconnexa</i>	Romanov & Kopyrina, 2014: 1245, 1246, Fig. 2

Note: Scribailo & Alix (2010) synonymized *C. inconnexa* and *C. hypnoides* with *C. vulgaris* despite the tylacanthous stem cortex of the former two.

did not confirm several important characters given in the first description (Allen, 1888): 1. Incomplete stem cortex consisting of non-conjoined primary tubes only with empty intervals between them; 2. imperfect, disconnected cortex on the upper corticated branchlet segment in the case of two corticated segments or one single imperfectly disconnectedly corticated segment; 3. formation of bract cells, bracteoles and gametangia between ecorticated segments of the branchlet. These characters are clearly visible in the original figures of the holotype (Allen, 1888). Moreover the holotype closely resembled a juvenile form of *C. contraria* A. Braun ex Kütz. as R.D. Wood noted in the confirmavit slip (<http://plants.jstor.org/specimen/ny00887705>). The emended description of *C. inconnexa* also emphasized that the holotype is an “apparently juvenile plant” (Wood & Imahori, 1964).

So, the typification of a juvenile specimen created the unavoidable ambiguity associated with this species due to the actual impossibility of describing several important characters of type specimens. These are characters of mature gametangia, ripe oospores and branchlets of mature plants. After reinvestigation of the holotype, *C. hypnoides* was considered as a synonym of *C. vulgaris* var. *inconnexa* f. *inconnexa* (Wood & Imahori, 1965). However, these specimens are also rather juvenile, as could be concluded from the description, drawings and critical comments about it (Robinson, 1906; Wood & Imahori, 1964, 1965). The holotype of *C. hippelliana* stored in I. Dąbska collection (Department of Hydrobiology, Adam Mickiewicz University, Poznań), studied and illustrated by Wood & Imahori (1964, 1965) are small plants 2-5 cm in length, partly ecorticate and heavily calcified which does not allow its reinvestigating (Maciej Gąbka, personal communication). Consequently, *C. inconnexa*, *C. hypnoides* and *C. hippelliana* holotypes are obviously “ambiguous and cannot be critically identified for purposes of the precise application of the name to a taxon” (McNeill *et al.*, 2012: 9.8), and the selection of an epitype for *C. inconnexa* is required. The best solution would be to select one of the mature North American specimens of *C. inconnexa* as the epitype. Moreover, the specimens referred to as *C. inconnexa* by other authors are nonidentical to the key morphological characters according to their descriptions. In particular, they might have an irregular haplo-diplostichous stem cortex or several undifferentiated branchlets present in a whorl (Corillion & Guerlesquin, 1971, 1972; Corillion, 1977). Strongly tylacanthous cortex of *C. hippelliana* was the main character for the conservation of this taxon as *C. vulgaris* var. *inconnexa* f. *hippelliana* (Wood & Imahori, 1965: 120), it was virtually within the range of emended diagnosis of *C. inconnexa* (Wood & Imahori, 1965: 119) and here the former taxon was synonymised with the last species. The gametangia formation near or between corticated segments only was the main reason for the conservation of *C. arrudensis* as *C. vulgaris* var. *inconnexa* f. *arrudensis* (Wood & Imahori, 1965: 120). It seems to be negligible difference. Therefore it was also referred to synonyms of *C. inconnexa* (Han & Li, 1994).

### Morphological characteristics

The long ecorticate segment and few short abbreviated corticated segments within branchlets are the main characters of *Chara inconnexa* distinguishing it from the closely related *C. contraria*. Other examples with similar taxonomic solutions are *C. filiformis* H. Hertzsch and *C. longiarticulata* F.S. Han. *Chara filiformis* is close to *C. contraria* and differs morphologically by branchlet features only (Krause, 1997). In fact, *C. inconnexa* differs from *C. contraria* with nearly the same characteristics as *C. longiarticulata* from *C. altaica* A. Braun in A. Braun & Nordst.

Table 3. Main differentiating characteristics of subgymnophyllous forms close to *Chara contraria* from *C. inconnexa* according to Wood & Imahori (1964, 1965) and Han & Li (1994)

Species	Differentiating characteristics from <i>C. inconnexa</i>
<i>C. arrudensis</i> Mendes	isostichous stem cortex, longer anterior bract cells, gametangia formation near corticated segments only
<i>C. brionica</i> Stapf	imperfect cortication of branchlet segments; bract cells, bracteoles and bracteoles also at ecorticate segments
<i>C. hippelliana</i> (Vilh.) Vilh.	larger oospores and narrow coronula of oogonia, relative length of anterior bract-cell and bracteoles
<i>C. hoveana</i> (R.D. Wood) Y.J. Ling, S.L. Xie & L.C. Qiu	imperfect cortication of branchlet segments; bract cells, bracteoles and bracteoles also at ecorticate segments
<i>C. intumescens</i> C.B.Rob.	comparatively developed posterior bract-cells, 1 to 4-fold longer than wide
<i>C. nitelloides</i> (A. Braun) R.D. Wood	relatively long cylindrical obtuse to apiculate end cell of branchlet, irregular 2-3-corticate stem cortex, longer anterior bract cells
<i>C. pistianensis</i> Vilh.	irregular branchlet cortication, i.e. from totally ecorticated to fully corticated branchlets, but with complete cortication, occasionally geminate spine cells, black oospores, nearly isostichous cortex
<i>C. scepusiensis</i> Fil.	imperfect cortication of branchlet segments

The other characters may be easily compared in matrix key to the charophytes species (van Raam, 2009).

*emend.* Hollerb. The first species was differentiated from the second with long ecorticate segments and two to three abbreviated short corticated segments (Han, 1964; Han & Li, 1994; Hollerbach & Krassavina, 1983); the other characteristics of *C. longiarticulata* fall inside the diagnosis of *C. altaica*, except for having three bracteoles and slightly wider oogonia and oospores.

These branchlets are not peculiar to *C. inconnexa* only, but are known for other species in combination with other distinctive characters (Table 3). Rather, *C. hippelliana* belongs to *C. inconnexa*. *Chara arrudensis* could be an extreme variant of stem cortex arrangement within a single species forming a gradient from isostichous *C. arrudensis* through isostichous – tylacanthous – strongly tylacanthous *C. inconnexa* to strongly tylacanthous *C. hippelliana*. The degree of primary tube prominence within a stem cortex varies within and between localities of *C. inconnexa*. The stem cortex can be strongly tylacanthous to isostichous. The exact determination of juvenile plants of these species is obviously problematic. The difficult differentiation of *C. inconnexa* and juvenile plants of *C. contraria* was also noted by Compère (1986) from the same habitat. Langangen & Breivik (2012) referred to *C. contraria* ecotypes with subgymnophyllous branchlets having a perfect or imperfect cortex. These are very similar to several species listed in table 2.

The imperfect cortication of particular branchlet segments or even a small number of corticated segments could be characteristic features of juvenile forms or the result from abnormal morphogenesis of plants growing in a suboptimal environment. So, the reassessment of the taxonomic value of branchlet characters and additional criteria is strongly needed. Another difficulty arose from the taxonomic reassessment of partly and imperfectly gymnohyllous forms. Several taxa descriptions were possibly based on juvenile and abnormal specimens. Therefore, the characteristics of early ontogenetic stages might be presented in diagnoses.

The development of partly gymnohyllous branchlets of *C. inconnexa* is rather a result of branchlet node absence within ecorticate segments (Frame, 1974). The term “undifferentiated segment” has therefore been suggested for the ecorticate part of a branchlet and “undifferentiated branchlet” for a whole branchlet without nodes. In the case of *C. inconnexa*, the complete absence of branchlet nodes, except basal nodes, is characteristic at the beginning of the growing season only (Frame, 1974). The lowest branchlet whorls and lowest stem internodes are ecorticate on juvenile plants of several corticated species of *Chara* or on their oldest parts of thalli germinated from oospores, bract cells and gametangia are absent on the branchlets of the lowest whorls of these plants (de Bary, 1875; Filarzsky, 1893; Oltmanns, 1904; Vilhelm, 1914; Goebel, 1918; Groves & Bullock-Webster, 1924).

So, it can be concluded that these first-developed branchlets are nodeless. The ecorticate branchlets without both bract cells and gametangia sometimes develop on depressed or obsolescent parts of thalli. They might also be nodeless.

### Morphological characters of Siberian specimens

The specimens referred to as *C. inconnexa* from the south of eastern Siberia did not look suppressed or unhealthy. The thalli were well-developed, rather tufted and not as elongated as in light-limited plants. The specimens were collected in a period favourable for the growth of charophytes. Gametangia were present on almost all corticated segments of well-developed branchlets. Therefore, we conclude that the studied specimens are not juvenile, abnormal or depressed.

Nevertheless, a notable regional peculiarity in the differentiation of *Chara inconnexa* and *C. contraria* in the south of eastern Siberia exists. Specimens of typical *C. contraria* with the short ecorticate branchlet segments have been found in only a few localities. The ecorticate segment is usually shorter or rarely, almost as long as the last corticated segment. The other form of *C. contraria* is common in this region. The relatively longer ecorticate segments usually constitute about one third or, more commonly, nearly half of the branchlet length. The relative length of the ecorticate segments is maximal in the lowest whorls and within branchlets with lower numbers of corticated segments. Specimens from these populations are most similar to *C. inconnexa* but the intergrades between *C. inconnexa* and *C. contraria* are actually absent. The common forms of *C. contraria* in the south of Eastern Siberia have 2-5 (usually 3 or 4) corticated, not abbreviated, segments.

### Oospores

The ornamentation of the oospore surface, reported for Chinese specimens of *Chara inconnexa* based on light microscopy observations, is granular (Han & Li, 1994). Incompletely mature oospores of *C. hippelliana* holotype are clearly granulated (Wood & Imahori, 1964). The oospore descriptions from China (Han & Li, 1994) and from our novel data therefore complete the diagnosis of *C. inconnexa*. The oospores of *C. hippelliana* holotype are dark brown (Vilhelm, 1914, 1923); they are completely black to the naked eye according to the protologue (Vilhelm, 1914), while the golden-brown oospores of the *C. hippelliana* holotype (Wood & Imahori, 1965) are immature (Wood & Imahori, 1964). The *C. inconnexa* specimens from Saskatchewan have dark brown to black oospores (Mann, 1994). Noteworthy specimens referred to as *C. hippelliana* from western Bengal (Ray & Chatterjee, 1986) differ from the diagnosis with longer black oospores, longer oogonia, larger

Table 4. Ornamentation pattern of *Chara contraria*, *C. nitelloides* and *C. inconnexa* based on SEM observations

<i>Species</i>	<i>Ornamentation pattern</i>
<i>C. contraria</i>	roughened due to the presence of irregular depressions and globular elevations (Mandal <i>et al.</i> , 2002) featureless at lower magnification, roughened by numerous depressions, pits and pores (John <i>et al.</i> , 1990) irregular depressions and globular elevations, resembles irregularly granulate to slightly papillate (Ahmadi <i>et al.</i> , 2012) granulate (Dyck, 1970 cited in: Leitch <i>et al.</i> , 1990) pustular (Romanov, unpublished)
<i>C. inconnexa</i>	pustular (orig.)
<i>C. nitelloides</i>	distinct elevations on the surface with the pore at the tip of elevation (Mandal & Ray, 1999)

antheridia and partly by having fewer oospore ridges and a lower degree of their prominence (9-10 prominent versus 10-12 faint ridges). These characteristics mostly agree with the description of *C. inconnexa*, except for having a smaller oospore width (297-313.5 versus 375-420  $\mu\text{m}$ ) and larger antheridia (396-412.5 versus 300-390  $\mu\text{m}$  in diameter). A comparison of oospores and gametangia dimensions is often problematic, however, due to changes during desiccation at herbarisation and subsequent treatment for study. It could be concluded that *C. inconnexa* has dark brown to black oospores. The ornamentation of ripe oospores of *C. contraria*, *C. nitelloides* and *C. inconnexa* revealed by SEM is compared in Table 4. The outer surface of oospores from the Krasnokamensk reservoir is close to the variant reported for *C. nitelloides* (Mandal & Ray, 1999).

## Distribution

*Chara inconnexa* has been reported in North America (Frame, 1974) for Canada: Ontario (Robinson, 1906; Wood & Imahori, 1964, 1965), Newfoundland (Mann, 1989), and Saskatchewan (Mann, 1994); and for the USA: Iowa (Allen, 1888; Robinson, 1906; Prescott, 1931; Wood & Imahori, 1964, 1965), Illinois (Ebinger & Vogel, 1977), Michigan (Rich *et al.*, 1971), and from the southwestern United States and northern Mexico without exact localities (Tindall, 1966).

In northern Africa the species occurs in Libya (Corillion, 1978; Compère, 1986), Egypt, Algeria, Mauritania (Corillion & Guerlesquin, 1971, 1972; Corillion, 1973), Tunisia (Corillion, 1977). It also known to exist in Madagascar (Corillion, 1968, 1973).

Within Eurasia, *C. inconnexa* has been reported in central Europe: the Czech Republic (Vilhelm, 1914, 1923) where no new specimens have been collected (Caisova & Gabka, 2009), Germany (Frame, 1974); in southern Europe: Portugal (Mendes, 1947, 1948; Wood & Imahori, 1964, 1965; Cirujano *et al.*, 2007, 2008); in Western Asia: Turkey (Altinayar & Onursal, 1982; Aysel, 2005; Anonymous, 2009; Barinova *et al.*, 2014, Romanov *et al.*, unpublished), Egypt on the Sinai Peninsula (Corillion & Guerlesquin, 1971); Eastern Asia: China, Hebei, Hunan and Shanxi (Su *et al.*, 1990; Han & Li, 1994; Ling *et al.*, 2000; Zhang *et al.*, 2008,

2010a, b; Han *et al.*, 2010; Shi *et al.*, 2012); and in southern Asia: India, western Bengal (Ray & Chatterjee, 1986; Pal & Chatterjee, 1988). The record of *C. inconnexa* for eastern Siberia is the first for northern Asia and Russia.

It can be concluded that *C. inconnexa* is a very rare species with a wide distribution range located mostly in temperate and subtropical zones of the Northern Hemisphere. Obviously, the exact outlining of its distribution range is highly complicated by the absence of specimens illustrations and descriptions in most reports.

## Ecology

*Chara inconnexa* is a freshwater species; which can grow in different types of water bodies with different environmental conditions. This species has been found in channels (Altinayar & Onursal, 1982; Compère, 1986), drainage ditches (Vilhelm, 1914), ditches, rice fields (Han & Li, 1994; Ling *et al.*, 2000), lakes (Wood & Imahori, 1965; Mann, 1989, 1994), strip mine ponds (Ebinger & Vogel, 1977), on riverbanks (Ray & Chatterjee, 1986), in the near-mouth stretches of rivers, river pools and reservoirs (this study). In the southwestern United States and northern Mexico, *C. inconnexa*-like species are typical inhabitants of cool-flowing alkaline waters in desert areas (Tindall, 1966). In Crooked Lake (Saskatchewan, Canada), it grew at a depth of 1.5-3 m between waves-affected margins and communities of submersed angiosperms extending to its edges; luxuriant beds of the species were found at a beaver-impacted part of the lake (Mann, 1994). It has been reported in cold springs (Zhang *et al.*, 2008; Shi *et al.*, 2012) and in a marl lake (Rich *et al.*, 1971), where it formed communities within a narrow band of 0.5 to 2 m near to the major lake outlet. Subgymnophyllous forms with perfect cortication on branchlet segments within taxa with a tylacanthous diplostichous stem cortex and diplostephanous stipulodes cannot not be directly attributed to any ecological factor or to growth in a particular ecoregion.

## CONCLUSION

The vast area and rare occurrences of *Chara inconnexa* point towards the opinion that this species might be an example of intraspecies variability. However, its “rarity” can be explained by the inclusion of these forms within variations of *C. contraria* as juvenile or abnormal or as a result of disregarding differential characters. The populations and monodominant communities of non-depressed well-developed plants referable to *C. inconnexa* do exist in distant localities as a definitive state of ontogenesis, based on reported and our own data. So, we propose to document all findings of these forms and to consider it as a separate species before the clarification of the taxonomic status of partly gymnohyllous forms. Molecular polymorphism and culture studies in combination with a study of morphogenesis are among the most promising tools for the delineation of subgymnophyllous species close to *C. contraria* and *C. vulgaris*.

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