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Nomenclature,Taxonomy, Reproduction and Life Cycle of the genus *Haematococcus*, *Haematococcaceae, Chlorophycea*

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Abstract

The motile, unicellular green alga *Heamatococcus* has come into prominence in recent years as a possible candidate for mass production for its red pigment, astaxanthin. The present paper summarizes the available information on nomenclature, taxonomy, morphology, life cycle and ultra-structure, which have so far been left out of the many reviews on this interesting alga.

Key words: *Haematococcus*, nomenclature, taxonomy, ultrasound, reproduction, life-cycle.

Introduction

Algae are known to accumulate in their cells under certain conditions, different types of carotenoids, such as β-carotene, astaxanthin, canthaxanthin, fucoxanthin etc. The unicellular motile green alga, *Haematococcus* was first recognized and described because of the striking red colour of the small water bodies inhabited by them. Among microalgae, species of *Haematococcus*, *Chlamydomonas, Trentepohlia, Euglena, Trachelomonas, Glenodinium* and many Chlorococcalean species (Czygan, 1968; Hagen et al., 1994; Lee and Zhang, 1999) have been shown to synthesize varying levels of this pigment. The alga is also commonly called “Blutregenalge" (blood-rain alga) as it frequently forms red coloured puddles, bird- baths, rock pools etc. just after rainfall (Czygan, 1970). The primary interest evinced in the study of this alga is due to this red pigment, which gets accumulated in aging cells when exposed to direct sunlight. With increasing recognition of the importance of astaxanthin in animal and human nutrition (Johnson and An, 1991), *H. pluvialis* became the object of study of a large number of investigations. The earlier studies concerning the location, mode of deposition and the conditions promoting the accumulation of pigment were not only because they were early studies dealing in detail on these and other aspects of taxonomy, nomenclature, morphology besides new reports from different geographical locations but also because of their academic value as pioneering studies (Almgren, 1966; Elliot, 1934; Pocock, 1960; Pringsheim, 1966). Iyengar and Desikachary (1981) also consolidate the taxonomic aspects along with descriptions of the erstwhile known taxa from literature along with notes on occurrence in India and south - east Asian genera.

There are already many detailed reviews on astaxanthin production in *Haematococcus* (Johnson and An, 1991; Lee and Zhang, 1999). Jeeji Bai and Kumaravel (2003) have briefly summarized the information on some important aspects of morphology, lifecycle and conditions of astaxanthin production. This review serves to add certain details on aspects of taxonomy, nomenclature, cellular morphology and ultra-structure in greater detail. Besides, studies on life cycle, modes of reproduction and different cell types reported are also included.

Nomenclature, Morphology and Life cycle

The genus *Haematococcus* was created by Agardh in 1828 with 2 species, *H. noltii* and *H. grevillei*. Both these species have later been shown to belong to other genera. At present, the following species of *Haematococcus* are recognized as validly described (Ettl, 1983):

1. *H. pluvialis* Flotow 1844 em. Wille 1903
2. *H. buetschlii* Blochmann 1886
   H. buetschlii var. *bahusiensis* Skuja 1956
3. *H. droebakensis* var. *droebakensis* Wollenweber 1907
   H. droebakensis var. *fastigiatus* Wollenweber 1908
   (Pringsheim, 1966, lists this as a synonym of *H. buetschlii*)
   H. droebakensis var. *danuvialis* Schmidt etUherkovich 1976
4. *H. capensis* Pocock 1960
   H. capensis var. *borealis* Pocock 1960 (listed only by Pringsheim, 1966)

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H. capensis var. *torpedo* Pocock 1960
H. capensis var. *piriformis* Pocock 1960

5. *H. zimbabwiensis* Pocock 1960

Droop (1956a) made the first detailed analysis of the nomenclature of *H. pluvialis*. According to him, out of the many synonyms, only *Volvox lacustris* Girod-Chantrans 1802, *Lepraria kermesiana* Wrangel 1823 and *Disceraea purpurea* Morren 1841 could be considered as possible synonyms. If indeed, they are so, the combinations *L. lacustris* and *D. lacustris* have priority over *H. pluvialis*. As *Lepraria* is insufficiently described and *Disceraea* has not been as widely used as *Haematococcus*, he has made the proposal that the genus *Haematococcus* be conserved. The International Code of Botanical Nomenclature conserved the genus *Haematococcus* Flotow (1844) and rejected that of Agardh and designated the species *H. pluvialis* Flotow as the type. *H. lacustris* (Girod-Chantrans) Rostafinski was listed as a synonym. Ettl (1983) listed *Sphaerella pluvialis* (Flotow) Wittrock 1883, *Sph. lacustris* (Girod) Wittrock 1883, *H. lacustris* (Girod) Rostafinski 1871 as synonyms of *H. pluvialis* and included *H. longistigma* Ettl 1954 also in this species. Almgren (1966) again analyzed the nomenclatural problem in detail and adopted the name *H. lacustris* following the view of Pocock (1960) who suggested that the description and drawings of Girod- Chantrans clearly showed that it was identical with *H. pluvialis* and the former had priority over the latter. The Cambridge and Texas culture collections and following them, many studies have also adopted the name *H. lacustris*.

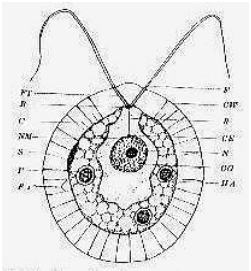
Droop (1956b) sesparated the two species, *H. buetschlii* and *H.droebakensis* into a separate genus *Balticola* based on the presence of papilla, number of pyrenoids and above all, what he considers as transverse plane of division as against the vertical division typical of *Haematococcus*. In a detailed study of several species collected from Africa, Pocock (1960) showed that the plane of division is indeed vertical and a rotation of the cell causes the delusion and therefore the new genus has been rejected by her and all later investigators. Ettl (1983) listed *Balticola* also as a synonym of *Haematococcus*. However, Almgren (1966) recognized this genus and gave a new key for the genera and species of *Haematococcaceae* in which he also included *H. zimbabwiensis* and *H. capensis* of Pocock (1960) in *Balticola*. Bourrelly (1972) includes the genus in a subfamily Haematococcoideae in the family Chlamydomonadaceae. Iyengar and Desikachary (1981) also recognize only the genus *Haematococcus* and provide a long list of references in their taxonomic treatise on Volvocales covering almost all aspects of taxonomy, morphology, ultrastructure, distribution and carotenogenesis. In this review, the view of Ettl is followed.

Generic description

Cells solitary, ovoid, ellipsoid or spherical with two equal flagella, with or without papilla, the cell wall separated from the protoplast by a wide space filled with a slimy substance; from the protoplast thin but firm strands extend to the wall; these strands branch out towards the wall in some species; the widely divergent flagellar bases are enclosed in tubular structures between the protoplast and the wall; the cup-shaped chloroplast is peripheral, irregularly perforate and reticulate with many pyrenoids or in the form of a narrow cylinder with basal and apical plates each with a pyrenoid; there are many (up to 60) contractile vacuoles scattered in the cytoplasm; the large nucleus is centrally placed with a nucleolus; the linear, club-shaped stigma is laterally placed half-way along the cell; aging cells show deposits of red haematochrome pigment in close proximity to the nucleus. During adverse conditions aplanospores are formed by the deposition of a thick wall close to the protoplast inside the outer cell wall, which later disintegrates.

Haematococcus pluvialis Flotow (Figure 1): The species is characterized by ovoid, ellipsoid or spherical cells, 34 – 37.5 µm in diameter (up to 5 µm broad and 63 µm long) with cell wall widely separated from the protoplast without a papilla but with an anterior protrusion in young cells; protoplast with a beak- like apex not reaching up to the wall, plasma strands branched; chloroplast cup shaped with thick membrane, with 6- 8 (up to 15) scattered pyrenoids, chloroplast not reaching into plasma strands; aplanospores spherical with smooth wall. Vegetative cells up to 63 µm long and 51 µm broad; aplanospores are spherical and reach up to 3 times the diameter of vegetative cells.

Figure 1: Composite diagram of the macrozooid stage; B: blepharoplast, C: chromatophore, CE: centrosome, CG: chromatin granules, CW: cellulose wall, F: flagellum, FT: flagellar tube, HA: hyaline area, N: nucleolus, NM: nuclear membrane, P: pyrenoid, PS: protoplasmic strands, R: rhizoplast,S: stigma (from Elliot, 1934)



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Ultrastructure

All studies on ultrastructure have been carried out on *H. pluvialis*. The main aim of these studies was to confirm the location and mode of deposition of haematochrome. The earliest study of Bowen (1965) [as quoted by Lang (1968)], showed the pigment to be deposited in numerous sacs in membrane-bound vacuoles. Wygasch (1966) also reported that the pigment deposits were located in vacuoles. Using two different methods of fixation and cells showing different stages of deposition- slight to heavy-, Lang (1968) proved the earlier studies wrong. Astaxanthin was deposited in the perinuclear cytoplasm, in close proximity to the nuclear envelope, in ER- ribosome associations as spheres and rods of different sizes and electron densities. They were formed neither within the ER-cisternae nor within vesicles. This was corroborated by the study of Santos and Mesquita (1984) who found pigment deposits of varied morphology, spherical, rod-shaped, filamentous and reticulate forms. There was no association with chloroplast lamellae although intense deposition caused the pressing of chloroplast to the cell membrane. They observed that the deposits often fused to form large bodies in old aplanospores. They also showed that unlike the case in other species where chloroplast lamellae also extended into the protoplasmic strands, in *H. pluvialis* the strands showed only mitochondria. In all these studies, the stigma was shown to be composed of a single layer of large granules of pigment deposits in the peripheral region of the chloroplast immediately beneath the envelope. Wygasch (1965) believed that the granules consisted of dense accumulations of astaxanthin pigment.

Reproduction and life cycle

Elliot (1934) recognized four types of cells: microzooids, macrozooids, palmella and haematocyst, the first two representing motile stages and the latter two the resting stages. The macrozooids are large more or less spherical with the typical widely separated wall whereas microzooids derived through excessive division of haematocysts are much smaller and ovoid in shape with closely appressed cell wall. Both forms are capable of rounding off to form resting cells with deposition of a new, thick and resistant cellulosic wall around the protoplast with the eventual disintegration of the original wall. Motile cells reproduce asexually by simple division along the longitudinal plane. Two successive divisions are common and occasionally three divisions are known to occur; the daughter cells ("zoospores") are fully formed within the parent cells, which remain motile till their flagella are dropped and the walls dissolve to release the daughter cells. The pyrenoids remain intact and divide by simple budding during cytokinesis.

Droop (1956b) reported dropping of flagella prior to cell division. *H. droebakensis* and *H. buetschlii* have prolonged motile phases whereas *H. pluvialis* has a short-lived motile phase. Young palmella cells usually enlarge and directly form haematocysts; they undergo a few divisions to give rise to a packet of aplanospores under favourable conditions, especially in cultures. The thick-walled haematocysts rest during adverse conditions and germinate after rains to produce motile cells (micro- or macrozooids, as described above). The haematocysts are known to remain viable for years.

Sexual reproduction by isogamy has been reported in *H. droebakensis*, *H. buetschlii* (Skuja, 1948) and *H. capensis* with 16-32 gametes in the first two and up to 256 gametes in the last one. These species produce gametes in motile phases (Pocock, 1960). The shape and size of gametes serve to distinguish the different species. Some species are heterothallic while others are homothallic. Iyengar and Desikachary (1981) give a comprehensive account of gametogenesis followed by isogamy in the 3 species with supporting figures from the original investigators. Gametic fusion was reported in *H. pluvialis* first by Peebles (1909) and then by Schulze (1927) but Elliot (1934) did not observe it and believed that division stages of microzooids could be mistaken for fusion. According to Hartmann (1943), *H. pluvialis* is homothallic whereas Droop (1956b) observed fusion only when different clones were mixed and hence considered it to be heterothallic.

Based on detailed observations, Elliot (1934) put forth a simple scheme of life cycle (Figure 2). The flagellate cells gave rise to palmella and these might divide to form flagellate cells or a pack of palmella cells or form haematocysts; the haematocysts in turn might give rise to a small number of macrozooids or a large number of microzooids according to the prevailing conditions. In most of the studies using single strains this has been the pattern of development with hardly any report of direct observation of fusion stages. It is therefore still not clear, whether *H. pluvialis* has sexual stage and if it has, whether it is homothallic or heterothallic.

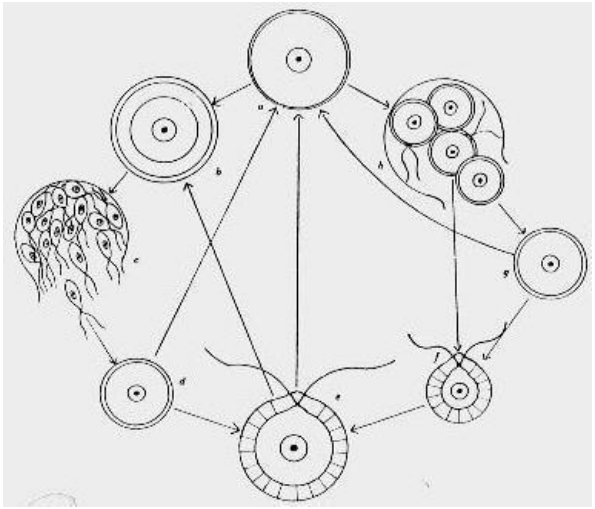

Figure 2: Life cycle of *Haematococcuspluvialis*; a,d,g) Palmella; b) Haematocyst; c) Microzooids; e) Adult cell; f) Macrozooid; h) Macrozooid / Palmella formation (from Elliot, 1934)

Lee and Ding (1994) observed that the akinetes release the zoospores when transferred to fresh media and the subsequent divisions are restricted to a maximum of 5 doublings. Our observations also confirmed this. The continued biomass increase through vegetative division of green motile cells required for mass culture purposes appears to be difficult to achieve at present. Green cells, which are capable of vegetative divisions, are transformed to non motile palmella stage or thick walled akinetes after undergoing 3-5 doublings. The palmella stage can revert back to motile stage after enlargement by undergoing a few divisions.

Elliot (1934) observed stages of nuclear division and cytokinesis in *H. pluvialis* and reported 20 – 30 chromosomes evenly distributed in the metaphase plate. He also observed the behaviour of centrosomes and rhizoplast of the neuromotor system during the mitotic division of motile cells. He showed that the pyrenoids divided equally or unequally like budding during cytokinesis. As mentioned earlier, Pocock (1960) also studied nuclear division in *H. pluvialis*. Recently, Lee and Ding (1994) studied the cell number vs. DNA content at different stages of the cell cycle and found a doubling of DNA content at 164 hrs. as against that at 112 hrs. They suggested a possible fusion of gametes during this time lapse. Triki et al. (1997) described an effective method of inducing gametogenesis in *H. pluvialis* (strain from culture collection of NIVA, Oslo, Norway). Starvation of cultures for at least one month followed by supply of fresh medium, induced gamete formation the very next day. Usually 32 or 64 gametes were formed which were less than 10 µm and moved very actively within the parent cell and after release they swam very rapidly and eventually settled and rounded off. Despite repeated trials, conjugation was not observed. The authors suggest cytological and cytogenetic studies to confirm meiosis and gametic fusion.

In Laboratory and open-air culture, we observed the release of 64 or more number of tiny, cylindrical motile cells from a single aplanospore. We could not observe fusion although it seems likely that they are indeed haploid gametes. Detailed studies on the chromosome number of the motile adult cells and these small “gametes” and their fused zygotes are required for ascertaining whether sexual reproduction is present in this alga.

Ecology and occurrence

The most typical habitats of *Haematococcus* sp. are small temporary water bodies, such as rainwater pools in cliffs and rocky shores of lakes and seas. This alga can be easily recognized by the blood red colour of the waters inhabited by them. One species has been reported to form the red snow of alpine regions. On drying up of such ephemeral pools, the red aplanospores (haematocysts) remain in the form of dry powdery incrustations or gelatinous aggregates. These get easily dispersed by wind or by birds. After prolonged resting during adverse conditions, they germinate afresh during spring rains. The genus has been reported from cool temperate zones, mainly from Europe and southern Africa.

Iyengar and Desikachary (1981) give a list of publications covering both descriptions and new reports from various regions of Europe, UK, Russia (as USSR) and the USA besides the African continent. Burchardt et al. (2006)

report the occurrence of *H. pluvialis* in an artificial pool in the university campus of a city in Poland. The many recent publications on astaxanthin biosynthesis in different strains isolated from different local water bodies and from various institutional culture collections suggest that this alga is quite widespread in occurrence in cooler regions of the world.

In the Indian continent Iyengar and Desikachary (1981) mention only one doubtful specimen from a tank in Trincomalie, Sri Lanka by Crow (1923). Another report by Kachroo (1960) from gut contents of anophiline mosquito larvae collected from water bodies of Damodar Valley. Susheela and Toppo (2006) observed massive development with different stages in many natural and man-made ponds of Himachal Pradesh. There is report of sporadic occurance of *H. pluvialis* (as *H. lacustris*) by Chakraborty et al. (2010) from brackish water region of Bengal from which the alga was isolated by them. This seems questionable because from the same source they also report *Dunaliella salina*. Bhosle and Dhumal (2012) report this alga as a constituent of freshwater phytoplankton from a water body in Kolhapur district of Maharashtra. The closely related species of *Sphaerellopsis* are frequently reported from different fresh water bodies of Maharashtra. It is likely that it occurs commonly in cooler parts of the country and evades detection due to its short life cycle. Devgoswami et al. (2011) have studied a local isolate from Assam.

Species of *Haematococcus* are generally reported from plains and high mountains sometimes as mass development. They are found even in small mud pots in green houses and in small containers, stone depressions and concrete basins, which get filled only during rains and dry up soon. While drying they form red deposition (autospores) on sided and bottom. The only report of *Haematococcus* from large water bodies are in the water used for urban supply where this alga is known to cause nuisance and chlorination eliminates this problem (Svorcova, 1964).

Conclusions

This review not only concerns problems of taxonomy and nomenclature but also includes aspects of reproduction and lifecycle. A good understanding of life cycle and conditions facilitating prolonged vegetative propagation will be of immense value in mass culture applications. Besides, factors which promote autospore formation, its enlargement and possibly also division need to be well understood for efficient exploitation of this interesting alga. Finally, the habitat ecology of the organism and geographical distribution also offer useful clues for mass propagation especially in warm tropics.

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