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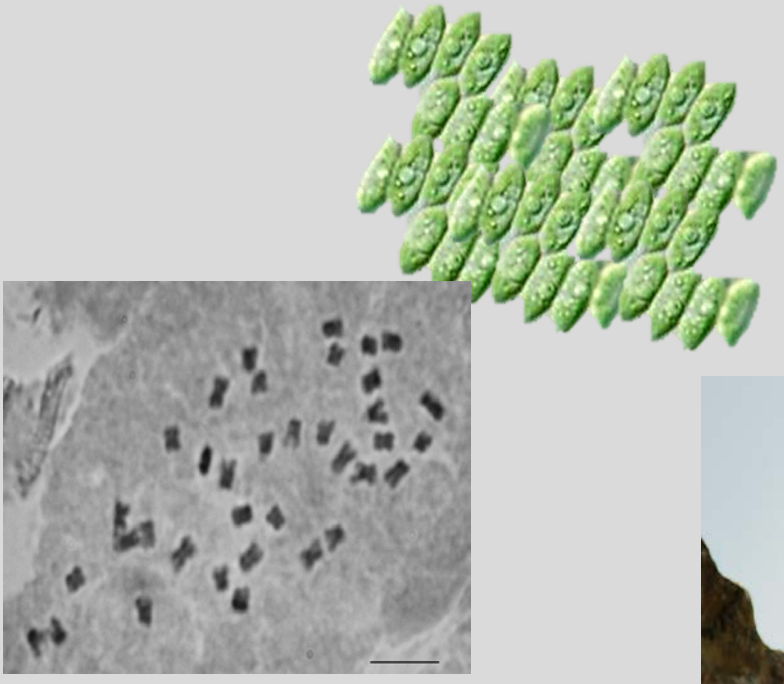

- ***Scenedesmus Acutus*** as biofuel reactor candidate
- Karyotype analysis of Common Cocklebur (*Xanthium strumarium L.*)
- A new bird species record for Turkey: Hume's wheatear (*Oenanthe albonigra Hume*, 1872)

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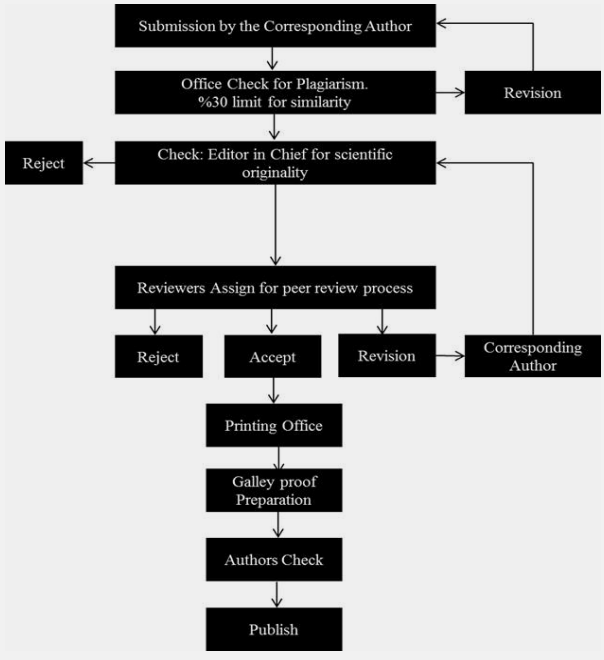

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Original Article

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Effect of nitrogen limitation on growth, total lipid accumulation and protein amount in *Scenedesmus acutus* as biofuel reactor candidate

Nur Agirman[1], Ahmet Kadri Cetin[1*]

Abstract

Objective: in this study, the changes in growth, protein and lipid amounts of *Scenedesmus acutus* which considering as biofuel producer microorganism in nitrogen-limited liquid media were investigated.

Material and Methods: The microalgal strain of *Scenedesmus acutus* was used which isolated from the Keban Reservoir in Eastern Anatolia, Turkey. Microalgal strain was grown in Jaworsky's medium. Algal cells were calculated by measuring the optical density at 680 nm using a visible density spectrophotometer. The total lipid content was determined using the Bligh and Dyer method. The total protein content was determined by the Lowry method.

Results: The results have shown that there is an inverse relationship between cellular growth, lipid amount and nitrogen concentration. *S. acutus* survived all applied nitrogen concentrations and increases were observed in the amount of its cellular lipid. It was determined that there was enough nitrogen in the nitrogen-limited media to support protein synthesis and cell growth of *S. acutus* and that the amount of lipid in the 50% nitrogen-limited media was 19.48% higher than that in the control group.

Conclusion: *S. acutus* survived in all the nitrogen concentrations tested (25% and 50% limited nitrogen in medium) and increases were observed in the amount of its cellular lipid. Significant increase in the amount of lipid in *Scenedesmus acutus* subjected to nitrogen stress suggests the idea that the microalga in question can be one of the potential organisms that can be used to obtain biofuel.

Keywords: *Scenedesmus acutus*, nitrogen limitation, protein, lipid accumulation, biofuel

Introduction

In recent years there have been many studies conducted on the discovery of renewable energy sources and their availability in various areas of everyday life. Biodiesel is one of the main renewable energy sources which is obtained from biological mass and has the potential to replace diesel fuel. Trapping carbon in biomass and allowing the restoration of global carbon balance, biomass energy is a more environmentally friendly option than other renewable energy sources (1). Microalgae have high potential as a source of renewable energy due to their high lipid content, rapid growth and low space requirements for their production. To this end, current studies have focused on identifying suitable biomass-producing species that provide higher energy output than traditional fossil fuels (2-4). Target species of choice for biomass production are those with known life cycles, fast cell division, high amount of protein and rich metabolite content.

Microalgae are much richer organisms in terms of photosynthesis and lipid content than terrestrial plants.

One of the main reasons for the increased biotechnological use of microalgae in recent years is that their lipid rate ranges from 20 to 50 % in general and even in some species, the dry weight of the lipid rate increases up to 80% (2,5,6).

Microalgae are known to undergo some morphological and biochemical changes as a survival strategy, especially under stress conditions. Many studies have been conducted on achieving high growth rates in algae species (7-10). Nutrient stress can strongly affect microalgal metabolism such as lipid content and photosynthesis. It was reported that high growth rate resulted in an increase in the biomass of microalgae whereas there was a high increase in the lipid content of microalgae grown under stress conditions (9,10).

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It was determined that there was a decrease in the rate of photosynthetic proteins in the cells of the green alga Chlamydomonas reinhardtii in nitrogen-limited media (11).

Temperature, light intensity and nutrient limitation such as nitrogen, iron and silicon are important factors affecting the amount and content of lipid in algae. The factor studied most often is the effects of nitrogen starvation on the amount of lipid (5). Nitrogen, which is the essential component of many macromolecules such as DNA, RNA, chlorophyll and protein, is known to be one of the most important nutrients for microalgae. Many studies report that nitrogen starvation leads to a decrease in photosynthesis and protein synthesis while an increase in lipid and carbohydrate synthesis (10,12,13). It was reported that the amount of nitrogen was 40% higher in Chlorella vulgaris grown in low-nitrogen media than in those grown in control media (14,15). It was also reported that the amount of lipid increased up to 7.9% in Nannochloropsis oculata and 5.9% in Chlorella vulgaris when the amount of nitrogen in the medium was decreased to 75% (16).

Chlorophyta members constitute a significant part of the algae used in biotechnological studies. *Scenedesmus acutus*, a member of Chlorophyta, is a freshwater alga. High protein, vitamin, mineral and lipid content of *Scenedesmus acutus* have made it biotechnologically important. It is considered one of the potential renewable sources in the future for alternative fuel production due to its rapid and high growth rate and high lipid content. Thus, this study observed the changes in the growth, and protein and lipid content of the green alga *Scenedesmus acutus* grown at different nitrogen levels.

Material and Methods

Algal Cultures and Media

The microalgal strain of *Scenedesmus acutus* used in this study was isolated from the Keban Reservoir in Eastern Anatolia, Turkey was grown in Jaworsky's medium consisting of 80 mg NaNO3, 36 mg Na2HPO4.12H2O, 20 mg Ca(NO3)2.4H2O, 12.4 mg KH2PO4, 50 mg MgSO4. 7H2O, 2.25 mg EDTAFeNa, 2.25 mg EDTANa2, 2480 µg H3BO3, 15.9 mg NaHCO3, 1390 µg MnCl2.4H2O, 1000 µg (NH4)6Mo7.4H2O, 40 µg biotin, 40 µg cyanocobalamin (B12) and 40 µg thiamin (B1). All media were sterilized at 121 °C for 15 minutes at 1 atmosphere pressure.

Two groups (treatment and control) were used for the experiments. The treatment group was from Jaworsky's medium with 25% and 50% nitrogen limitations and the original Jaworsky's medium was used as the control group.

Scenedesmus acutus was inoculated into 250-ml Erlenmeyer flasks containing 100-ml Jaworsky's medium. The Erlenmeyer flasks were incubated in a climate cabinet at 23 ±1 °C, light density 55 µmol photon m-2s-1, for 16-h light followed by 6-h darkness.10-ml samples from the media that reached a certain density were inoculated into the control and nitrogen-limited media. The inoculated control and 25% and 50% nitrogen-limited media were incubated at 23 ±1 °C, light density 55 µmol photon m-2s-1, for 16-h light followed by 6-h darkness. The Erlenmeyer flasks were shaken three times a day without CO2 addition. The analyses of the number of *S. acutus*, and protein and lipid content of the control and 25% and 50% nitrogen-limited media were performed for ten days in three repetitions.

Growth Measurement

S. acutus was counted under a microscope using a plankton counting chamber. Meanwhile, the same samples were examined daily using a visible spectrophotometer at a wavelength of 680 nm. Algal cells were calculated by measuring the optical density at 680 nm using a visible density spectrophotometer. The measurements on the spectrophotometer were compared with microscopic counts. A standard curve relating optical density was generated and used to calculate the numbers of individuals based on optic density. The calculations were performed in three repetitions.

Determination of Total Lipid

The total lipid content was determined using the Bligh and Dyer method (17). A mixture consisting of 40-ml methanol and 80 ml chloroform was added onto a 0.2-gr sample on which 20-ml CaCl2 (0.4%) was then added. The mixture was filtered through a filter paper and kept overnight in the dark. The next day, methanol/water phase was removed using a separating funnel and chloroform was evaporated in a 60 °C water bath. The remaining mixture was kept in a 90ºC drying-oven for 1 hour to evaporate the chloroform completely and then weighed.

Determination of Total Protein

The total protein content was determined by the Lowry method (18). 0.1-ml DOC solution was added onto 1-ml sample and the sample was kept at room temperature for 10 minutes. Afterwards, 0.1-ml TCA was added onto the sample which was, then, centrifuged at 7500 rpm for 10 minutes. After the removal of the supernatant, 1-ml Lowry solution was added to the precipitate and the precipitate was kept at room temperature for 20 minutes. Later on, 1-ml folin reagent was added to the sample, which was, then, kept for 30 minutes. Lastly, a standard curve was made by plotting absorbance at 750 nm and the results were evaluated based on the standard curve.

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Results and Discussion

Effect of nitrogen limitation on growth of *S. acutus*

Studies on the determination of factors affecting the cellular growth and metabolite content of algae are important nowadays. This study investigated the effects of different nitrogen concentrations on the cellular growth, and protein and lipid content of *Scenedesmus acutus*. Figure 1 shows the growth of *S. acutus* in the modified Jaworsky's medium at different nitrogen concentrations. The 10 day observation period after the day of inoculation indicates that the rate of increase in cell number is 325.95%in the control group, 335.38% in the 25% nitrogen-limited media and 410.50% in the 50% nitrogen-limited media. Figure 1 demonstrates that the rate of increase in cell number in the 50% nitrogen-limited media is significantly higher than those in the other media. Nitrogen is known to be the most important nutrient element for microalgae since it is necessary for the synthesis of organic materials such as protein, chlorophyll and nucleic acids. This study yields that the nitrogen limitation changes the rate of cell division to some degree. It is stated that the growth rates of microalgae in nitrogen-limited media are high (14,19-21). The results of this study also reveal that the cell growth rate of *S. acutus* is higher in the nitrogen-limited media than in the other media and that there is an inverse relationship between the growth rate of *S. acutus* under nitrogen stress conditions and the nitrogen concentration.

The Effect of nitrogen limitation on the protein amount

Proteins are the basic building blocks of living organisms. Approximately 50% of the dry weight of *S. acutus* is composed of proteins. Figure 2 shows the changes in the amount of protein in the control group and the nitrogen-limited media. The amount of protein, which was 44.145 µg/ml in Jaworsky's medium (control group) on the day of inoculation, regularly increased and reached 72.503 µg/ml on day 10 (Figure 2). The amount of protein, which was 44.145 µg/ml in the 25% nitrogen-limited media on the day of inoculation, regularly increased and reached 67.95µg/ml on day 8, however, started to decrease the following days and reached 63.048µg/ml on day 10. A similar situation was observed in the 50% nitrogen-limited media as well. The amount of protein in the 50% nitrogen-limited media continued to increase until day 8 (64.60µg/ml) and then started to decrease and reached 61.30µg/ml on day 10 (Fig.2). The comparison of Jaworsky's medium and the nitrogen-limited media reveals that the increase in protein in the latter is lower than in the former. During the 10-day experiment, rates of increase in protein were 64.238%, 42.820% and 38.860% in the control group, 25% nitrogen-limited media and 50% nitrogen-limited media, respectively. There is approximately a 25% decrease in the amount of protein in the nitrogen-limited media compared to the control group. There is an inverse correlation between the amount of nitrogen in the liquid media and the amount of protein.

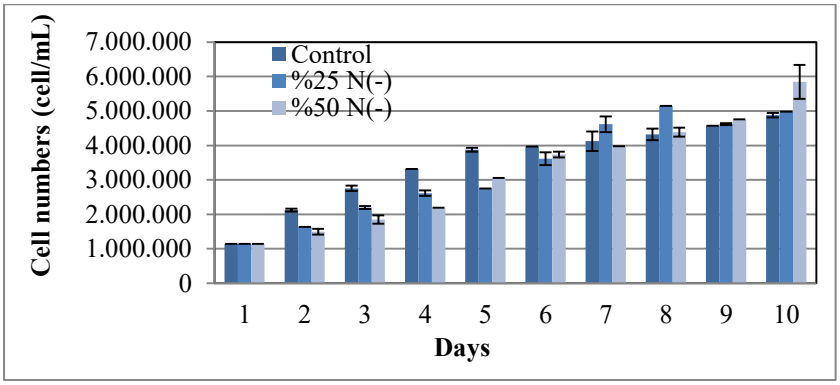

Figure 1: Effect of nitrogen limitation on growth of *S. acutus*

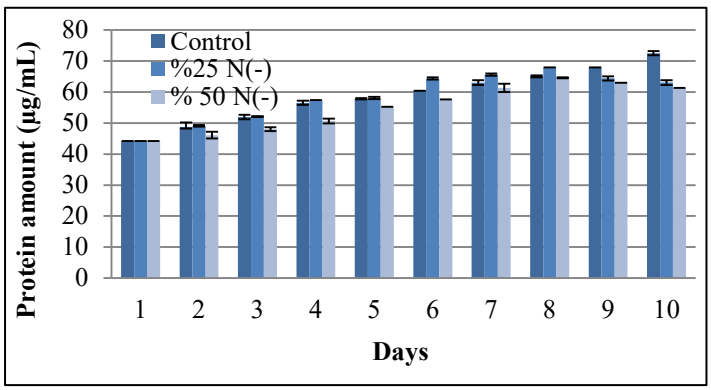

Figure 2: The effect of nitrogen limitation on protein amount of *S. acutus*

The effect of nitrogen limitation on the lipid amount

Lipid accumulation in microalgae can be significantly increased by nitrogen limitation in a culture medium (5,22,23). This study investigated the changes in the amount of lipid of *S. acutus* by nitrogen limitations in liquid nutrient medium. The amount of lipid, which was initially 7.7%, reached 11.4% in the control media at the end of the study period. The results of the experiment show that the increase in the amount of lipid in the control media is 48.05%. The amount of lipid of *S. acutus* grown in the 25% nitrogen-limited media was 7.7% on the day of inoculation and reached 12.7% on day 10 with a 64.93% rate of increase. However, the increase in the amount of lipid in the 50% nitrogen-limited media was significantly high. The amount of lipid in these media reached 12.9% and the rate of increase was 67.53% more than it was on the day of inoculation. The results indicate that the amount of nitrogen in the culture medium has a significant effect on the lipid content of *S. acutus* and that there is an inverse relationship between the amount of nitrogen in the culture medium and the amount of lipid (Fig.3).

This study shows that the biochemical composition of microalgae can be manipulated by changing the physical and chemical parameters of the medium. Many factors such as nitrogen deficiency in the medium, phosphate limitation, temperature and pH are known to affect lipid the content of microalgae (2,24,25). Nitrogen deficiency leads to stress conditions for all organisms because nitrogen is the main component of proteins and nucleic acids in living cells. When inoculated into a low-nitrogen containing medium, *Chlorella vulgaris* accumulate more total lipid in their cells. Widjaja *et al*. report that the ratio of total lipid and triglycerol in *C. vulgaris,* a freshwater microalga, significantly increased when grown in a nitrogen-depleted medium (26). Limited cell division, an increase in cell volume, and in lipid and carbohydrate synthesis were observed in *Chlamydomonas reinhardii* and *Scenedesmus subcapitatus* grown in nitrogen-limited media (27).

All studies demonstrate that the lipid content of microalga cells can be enhanced by nitrogen limitation.

The results of this study also confirm that the lipid content of *S. acutus* is significantly affected by the amount of nitrogen in the liquid media and that nitrogen deficiency leads to a decrease in the growth rate of and an increase in lipid accumulation in *S. acutus*. There are some proposals to account for the lipid accumulation of microalgae under nitrogen deficiency conditions. Botham and Ratledge argue that when nitrogen is depleted due to high energy load (ATP/AMP ratio), glucose conversion into lipids is triggered (28). Some researchers maintain that nitrogen deficiency promotes the conversion of excess glucose into lipid and leads to a higher rate of lipid transformation than that of cell division under autotrophic or heterotrophic culture conditions (29-31). One of the mechanisms suggested for the explanation of lipid accumulation in microalgae under nitrogen deficiency conditions is based on the premise that chloroplast nitrogen is transported using1.5-bifosfat carboxylase/oxygenase which leads to the mobilization of the lipid in chloroplast membranes (32). Another mechanism suggested for the explanation of lipid accumulation in microalgae grown under N deficiency conditions is attributed to mobilization of lipid from chloroplast membranes due to repositioning of the chloroplast nitrogen by using 1,5-bisphosphate carboxylase/oxygenase. Sheehan, et al. hypothesize that the reason for the increase in lipid content is that nitrogen depletion leads to the inhibition of cell division without a gradual decrease in lipid production which results in accumulation of fat in cells (33). Other researchers suggest that lipid accumulation may be related not only to high levels of lipid-synthesizing enzymes under nitrogen deficiency conditions but also to the inhibition of cell growth and reproduction by the operation of lipid-accumulating special enzymes (30,34). It is also maintained that many microalgae are able to adapt their metabolic pathways to store lipid in high amounts under nitrogen deficiency conditions (5,12,35,36).

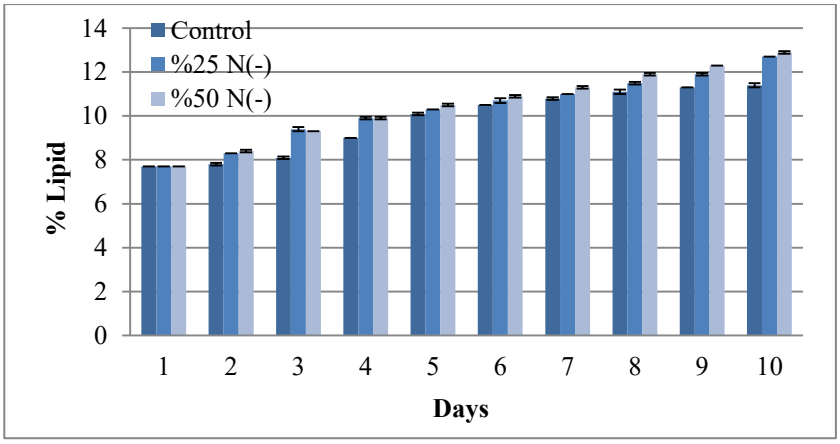

Figure 3: The effect of nitrogen limitation on lipid amount of *S. acutus*

Sheehan et al. reported that the nitrogen deficiency conditions led to a shift in the carbon flux from protein synthesis to lipid synthesis which resulted in an increase in lipid content in microalgae (33). However, nitrogen limitation does not always lead to lipid accumulation. *Achnanthes breviceps* and *Tetraselmis* sp. grown under nitrogen deficiency conditions were observed to have accumulated carbohydrates (28,37). This indicates that lipid accumulation due to nitrogen deficiency is species specific. The decrease in the amount of protein and the increase in the amount of lipid in *S. acutus* subjected to nitrogen stress indicate that the microalga in question adapts its metabolic pathways to store lipid under nitrogen deficiency conditions.

Conclusions

This study examined the changes in growth, and protein and lipid amounts of *S. acutus* grown in liquid media subjected to nitrogen stress. The results show that there is an inverse relationship between cellular growth, lipid amount and nitrogen concentration. *S. acutus* survived all the nitrogen concentrations tested and increases were observed in the amount of its cellular lipid. It was determined that there was enough nitrogen in the nitrogen-limited media to support protein synthesis and cell growth of *S. acutus* and that the amount of lipid in the 50% nitrogen-limited media was 19.48% higher than that in the control group. Significant increase in the amount of lipid in *Scenedesmus acutus* subjected to nitrogen stress suggests the idea that the microalga in question can be one of the potential organisms that can be used to obtain biofuel.

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Ethical issues: All Authors declare that Originality of research/article etc... and ethical approval of research, and responsibilities of research against local ethics commission are under the Authors responsibilities. The study was conducted due to defined rules by the Local Ethics Commission guidelines and audits.

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Original Article

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Karyotype analysis of Common Cocklebur (*Xanthium strumarium L.*)

Gulden Dogan[1]* Yasar Kiran[1]

Abstract

Objective: The purpose of this study is to determine the mitotic chromosome number, morphometric parameters, and karyotypes of *Xanthium strumarium* and make contributions to other multidisciplinary studies on the genus.

Material and Methods: The seeds were germinated on moist filter paper in Petri dishes at 25°C. Actively growing root tips were pre-treated with aqueous paradichlorobenzene for 4h at room temperature. Then, the root tips were fixed with acetic alcohol (1:3 glacial acetic acid–absolute ethanol) for at least 24 h at 4°C, hydrolysed in 1 N HCl at 60°C for 3 min, then rinsed in tap water for 3–5 min. Finally, they were stained in Feulgen for 1 h and mounted in 45% acetic acid. Digital microphotographs from at least five well-spread metaphase plates were taken using an Olympus BX51 microscope and were recorded with an Olympus Camedia C-4000 digital camera.

Results: The chromosome number is determined as 2n = 36 for this taxon. The karyotype consists of 16 median region (m) and 2 submedian region (sm) chromosomes. The metaphase chromosome length ranges from 2.30 to 4.03 µm, longest to shortest chromosome ratio is 1.7:1.1, total karyotype length (TKL) 54.76 µm and the karyotype symmetry is type 1A.

Conclusion: The results of this study showed that the chromosome number of Xanthium strumarium is 2n=36. Satellites were not observed in the karyotype of this species. Identifying the chromosome number of this species in this study provides a base for biosystematic studies.

Keywords: Chromosome number, *Xanthium*, Karyotype Analysis.

Introduction

The genus *Xanthium* L. belongs to the family Asteraceae. The members of this genus are distributed globally, however are most frequently in to be found tropical and sub-tropical regions (1). They are wide-spread in America, Canada, Mexico, Malaysia, Indonesia and India. The taxonomic study of the members of this genus is difficult and confusing. For example, Caius reported that the genus *Xanthium* includes 25 species (2). However, according to Weaver and Lechowich, there are 20 species belonging to this genus. Thus confusion about the species number of this genus still exists (1). Love and Dansureau revised the genus *Xanthium* and reduced the number of species to only 2 (*X. strumarium* L. and *X. spinosum* L.). They mentioned that due to phenotypic plasticity in a number of features, the members showed different phenotypes and creating confusion regarding their taxonomic rank (3). Prain described that the 2 species *i.e. X. strumarium* and *X. spinosum* were found in undivided Bengal (4). However, Oudhia and Dixit (1994) reported *X. indicum* and *X. strumarium* from India (5). In Turkey, only two species, namely *X. strumarium* and *X. spinosum* have so far been reported. *X. strumarium* species found in Turkey has two subspecies (subsp. *strumarium* and *cavanillesii*) (6).

The common names are cocklebur, burr, sheep burr, etc. (7-10). The plant is an annual 30-120 cm in height and is a short-day plant that flowers in July-August. Each cocklebur bur contains two seeds. The seeds are covered by a hard green husk with hooked spines (11). The whole plant (*X. strumarium*), especially its leaf, root and fruit, has been used in traditional medicine for the treatment of rhinitis, malaria, rheumatism, tuberculosis, cancer, and ulcers (12-15). Previous studies indicated that plants of the Asteraceae family are characteristically rich in sesquiterpene lactones, an important class of terpenoids, and the *Xanthium*'s species are rich in such medicinal ingredients.

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The pharmacological properties of *X. strumarium* are largely attributed to the presence of xanthanolides (a class of sesquiterpene lactones), which have been reported to possess antifungal, antibacterial, and cytotoxic activities, and exhibit a growth inhibitory activity against insects (16-22).

The plant is used in classical homeopathy (23) and is officially recognized in China and several other countries. The preparation Adenostop is manufactured from cocklebur in Romania and is used to treat prostate adenoma. High anticancer activity of cocklebur (for breast, lung, stomach, and colon cancer) was recently reported (24). This plant is used as a medicine for curing nasal sinusitis, headache, urticaria and arthritis. It has also been reported to possess curative effects against chronicbronchitis, chronic rhinitis, allergic rhinitis, lumbago and other ailments (25) and is used by various native American tribes to relieve constipation, diarrhea and vomiting (26).

The purpose of this study is to determine the mitotic chromosome number, morphometric parameters, and karyotypes of *Xanthium strumarium* and make contributions to other multidisciplinary studies on the genus.

Material and Methods

Plant material was collected from natural habitats during the fruiting season in Elazig in 2015. Voucher specimen was deposited at the Firat University Herbarium (FUH). Karyological studies were conducted on meristematic cells obtained from the root tips.

The seeds were germinated on moist filter paper in Petri dishes at 25°C. Actively growing root tips were pre-treated with aqueous paradichlorobenzene for 4h at room temperature. Then, the root tips were fixed with acetic alcohol (1:3 glacial acetic acid–absolute ethanol) for at least 24 h at 4°C, hydrolysed in 1 N HCl at 60°C for 3 min, then rinsed in tap water for 3–5 min.

Finally, they were stained in Feulgen for 1 h and mounted in 45% acetic acid. Digital microphotographs from at least five well-spread metaphase plates were taken using an Olympus BX51 microscope (Olympus Optical Co. Ltd., Tokyo, Japan), and were recorded with an Olympus Camedia C-4000 digital camera (Olympus Optical Co. Ltd., Tokyo, Japan). The short arm (s), long arm (l) and total lengths (tl) of each chromosome were measured and the relative lengths, arm ratios, and centromeric indices were determined from images of selected cells. Chromosomes were classified according to the nomenclature of Levan et al. (27). The intra-chromosomal asymmetry index (A1) and the inter-chromosomal asymmetry index (A2) followed those of Romero-Zarco (28). The karyotype symmetry nomenclature followed Stebbins (29). Also, relevant literature the online chromosome number databases, Index to Plant Chromosome Numbers (IPCN) (30) and Index to Chromosome Numbers in Asteraceae (31) were checked.

Results and Discussion

The results of this study showed that the chromosome number of Xanthium strumarium is 2n=36. Karyotype analysis of this species to reveal the many values were calculated. The number of somatic chromosome, ploidy level, karyotype formula, chromosome length range, total karyotype length (TKL), Stebbine C and asymmetry indexes (A1, A2) are presented in Table 1; relative length, arm ration, centromeric index, type, in Table 2.

Haploid ideograms of *X. strumarium* has been shown in Fig. 1 and metaphase chromosomes in Fig. 2. The chromosome number is determined as 2n = 36 for this taxon. The karyotype consists of 16 median region (m) and 2 submedian region (sm) chromosomes. The metaphase chromosome length ranges from 2.30 to 4.03 µm, longest to shortest chromosome ratio is 1.7:1.1, total karyotype length (TKL) 54.76 µm and the karyotype symmetry is type 1A. Satellites were not observed in the karyotype of this species.

Table 1. Somatic chromosome number, ploidy level, karyotype formula, chromosome length range, total karyotype length (TKL), asymmetry indexes (A1, A2) of Romero Zarco (1986) and symmetry classes (SC) of Stebbins (1971) of *Xanthium strumarium*.

Taxon	2n	Ploidy level	Karyotype formula	Chromosome length range (µm)	TKL (µm)	A1	A2	SC
Xanthium strumarium	36	4x	16m+2sm	2.30-4.03	54.76	0.21	0.15	1A

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Table 2. Karyomorphological parameters of *Xanthium strumarium*

Xanthium strumarium				
Pair No	Relative Length	Arm Ration	Centromeric Index	Type
1	7.36	1.16	0.46	m
2	6.89	1.20	0.45	m
3	6.43	1.17	0.45	m
4	6.40	1.38	0,41	m
5	6.16	1.26	0.44	m
6	5.88	1.19	0.45	m
7	5.77	1.44	0.40	m
8	5.67	1.23	0.44	m
9	5.49	1.25	0.44	m
10	5.43	1.78	0.35	sm
11	5.15	1.11	0.47	m
12	5.11	1.22	0.44	m
13	5.01	1.32	0.43	m
14	4.99	1.78	0.35	sm
15	4.94	1.25	0.44	m
16	4.62	1.18	0.45	m
17	4.42	1.15	0.46	m
18	4.21	1.20	0.45	m

Meiotic chromosome number is n=18 this species was reported in the literature (32-36). Besides, in de book of "Flora der Schweiz und angrezender Gebiete" reported that chromosome number of *X. strumarium* n=18.

2n=36 chromosomes of this species was reported in the literature (37-54). Therefore, the present count confirmed the earlier reports on 2n chromosomes number.

Conclusion

According to our knowledge, chromosome number and morphology report for *Xanthium strumarium* does not exist. Identifying the chromosome number of this species in this study provides a base for biosystematic studies.

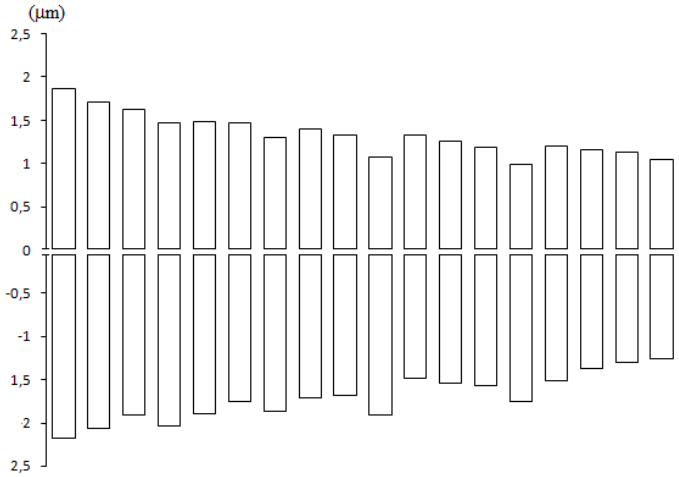

Figure 1. Haploid idiograms of **Xanthium strumarium**.

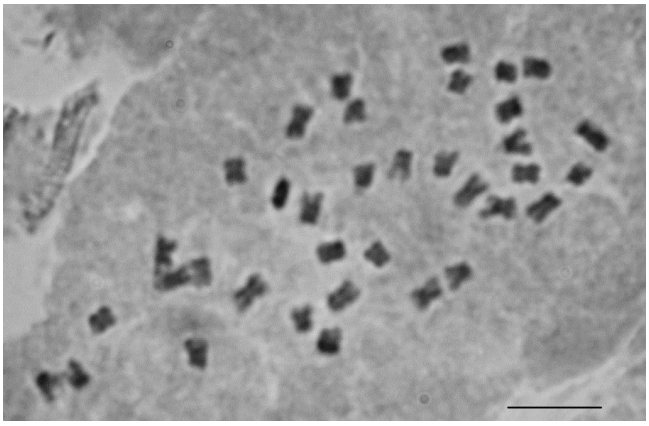

Figure 2. Metaphase chromosomes of **Xanthium strumarium.** Scale bar=10 µm

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Author's Contributions: GD, YK: Collecting of data, writing and revision of article,

Ethical issues: All Authors declare that Originality of research/article etc... and ethical approval of research, and responsibilities of research against local ethics commission are under the Authors responsibilities. The study was conducted due to defined rules by the Local Ethics Commission guidelines and audits.

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Original Article

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A new bird species record for Turkey: Hume's wheatear (Oenanthe albonigra Hume, 1872)

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Abstract

Objective: The aim of this study is to report Oenanthe albonigra,the new record to the avifauna of Turkey.

Material and Methods: This report includes the first record of Hume's wheatear in Turkey which has been observed around the Historical Hoşap Castle in Van province.. The observations were made out between 2014 and 2016. It is first recorded in 2014. And for 2015 and 2016 field studies have been planned. The field work was carried out in the months of May, June, July, August and September for each year. Field work was done one day per month. In total, 15 observations were carried out during these three years

Results: The species Hume's wheatear was observed for the first time inside the borders of Turkey in June 2014. The first detection of the species was achieved at Hoşap Castle, which belongs to the Gürpınar district of Van province. At the first encounter, two pairs (four individuals) were seen. The species was seen at 11 locations in total. The species were observed in the area in the period between May and September each year. In total, 6 individuals were recorded in 2015, and 10 individuals were recorded in 2016

Conclusion: By this study, it was revealed that the species Hume's wheatear was detected during the observations in Van province for the first time in Turkey. Therefore, this species is a new record for birds of Turkey. The number of bird species in Turkey has been increased to 513 by this result. As a result of these observations, it was concluded that the species is a Summer Migrant for the area, and they incubate.

Key Words: Hume's wheatear, Oenanthe albonigra, Birds, Van, Turkey, First record

Introduction

The genus Oenanthe, which is a member of Passeriformes order in the family Muscicapidae, consists of 22 species all over the world. 16 of these species are distributed in Palearctic and Afrotropic zones (1).

Hume's wheatear was named by Allan Octavian Hume for the first time in Pakistan in 1872 as Saxicola alboniger. The synonym Oenanthe albonigra was also expressed in later periods. The species distribution seems to include Iran, Iraq, Afghanistan, Bahrain, India, Kuwait, Oman, Pakistan, Qatar and United Arab Emirates The species is listed in the LC (Least Consern) criteria of IUCN.

There is no information about the observation of the species in Turkey in the studies of Birds of Turkey made by Ergene, Vielliard, Kumerlove, Kiziroğlu, Clements, Kirwan and others from 1900's to our time (2-6).

Additionally, there is no information about the distribution of the species in Turkey and further north in Heinzel and Svensson's field guides (7-8). Before that record, number of species belonging to the genus Oenanthe was 11 in Turkey (6). As for Van province, there were 7 records regarding Oenanthe species (9-10). This new type of record is important because it will contribute to Turkey's avifauna.

Materials and Methods

The first record for Hume's wheatear in Turkey was made in and around the Historical Hoşap Castle in Van province. The Castle is approximately 40 km far from the center of Van, 55 km in the east, and 160 km in the south from Iran border as a beeline. It is in a distance of 125 km from Iraq border in south (Fig. 1).

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The observations were made out between 2014 and 2016. It is first recorded in 2014. And for 2015 and 2016 field studies have been planned. The field work was carried out in the months of May, June, July, August and September for each year. Field work was done one day per month. In total, 15 observations were carried out during these three years.

Dobinson method was utilized for field work (11). This method includes the surveillance of a vantage point and transects observations on a particular line with optical equipment. The coordinates of the species' visual contact points, number of individuals and videos were recorded.

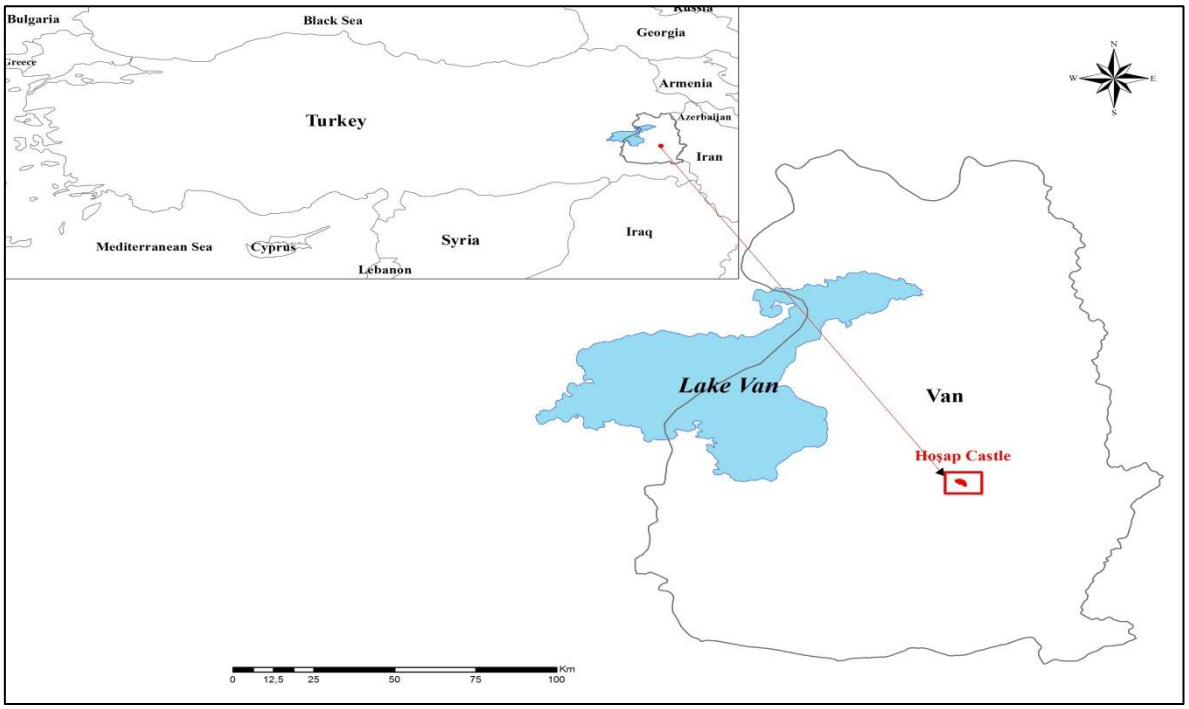

Figure 1: The location map of research area

Table 1: The coordinates of detection for the species in the research area

Year	Number of Individuals	UTM	X	Y
2014	2	38 S	395175 E	4241687 N
2014	2	38 S	395236 E	4241684 N
2015	2	38 S	395254 E	4241709 N
2015	1	38 S	395146 E	4242007 N
2015	1	38 S	395058 E	4242190 N
2015	2	38 S	395685 E	4241297 N
2016	3	38 S	396058 E	4240644 N
2016	2	38 S	395266 E	4241639 N
2016	2	38 S	395294 E	4241675 N
2016	2	38 S	393875 E	4241766 N
2016	1	38 S	393661 E	4241742 N

Table 2: Numerical distribution of individuals of the species in the research area based on years

Year/Month	May	June	July	August	September	Total
	Number of Individuals					
2014	-	4	-	-	-	4
2015	1	1	2	2	1	6
2016	2	2	1	3	2	10
Total	**3**	**7**	**5**	**3**	**2**	**20**

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Results

The species Hume's wheatear was observed for the first time inside the borders of Turkey in June 2014. The first detection of the species was achieved at Hoşap Castle, which belongs to the Gürpınar district of Van province. At the first encounter, two pairs (four individuals) were seen.

Additionally, surveillance was conducted in the first observation point and its vicinity in 2015 and 2016. The species was seen at 11 locations in total. The coordinates of these locations are shown in Table 1.

The species were observed in the area in the period between May and September each year. In total, 6 individuals were recorded in 2015, and 10 individuals were recorded in 2016 (Table 2, Fig. 2).

It was observed that the individuals of the species which made nests in the area carried food to their nests. The individuals carrying food in their mouths to their nests were followed and it was seen that they prefers hollow spaces in rocks in the castle as nesting places (Image 1).

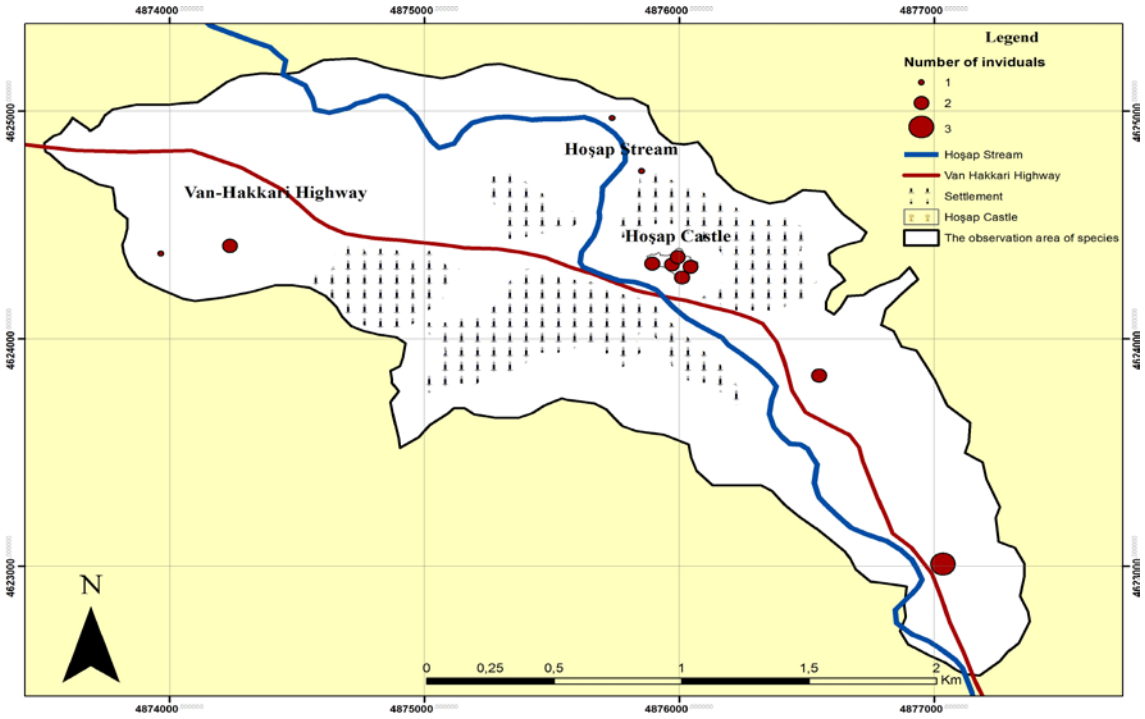

Figure 2. The distribution map of the species in the research area

Image 1: Images of the Hume's wheatear (*Oenanthe albonigra*) species in the research area, whereas the picture on the right shows individual carrying mouthfuls of food to its nest.

Conclusions

By this study, it was revealed that the species Hume's wheatear was detected during the observations in Van province for the first time in Turkey. There is no information about the species' distribution inside Turkey before 2014 within the available studies. Therefore, this species is a new record for birds of Turkey. The number of bird species in Turkey as been increased to 513 by this result (6).

Field work was conducted over three years to reveal the species' status in the surveyed area. As a result of these observations, it was concluded that the species is a Summer Migrant for the area, and they incubate.

The number of individuals belonging to the species in the area showed an increase by years. Additionally, the boundaries of its distribution area showed an extension in all directions from the point that it was seen for the first time. The species prefer rocky and barren districts in the area.

Based on the result of this study, it was proposed to the Ministry of Forestry and Water Affairs to include the species in monitoring program.

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Conflict of Interest: The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author's Contributions: EA: Field studies **OA, EA, IK:** Collecting of data, writing and revision of article,

Ethical issues: All Authors declare that Originality of research/article etc... and ethical approval of research, and responsibilities of research against local ethics commission are under the Authors responsibilities. The study was conducted due to defined rules by the Local Ethics Commission guidelines and audits.

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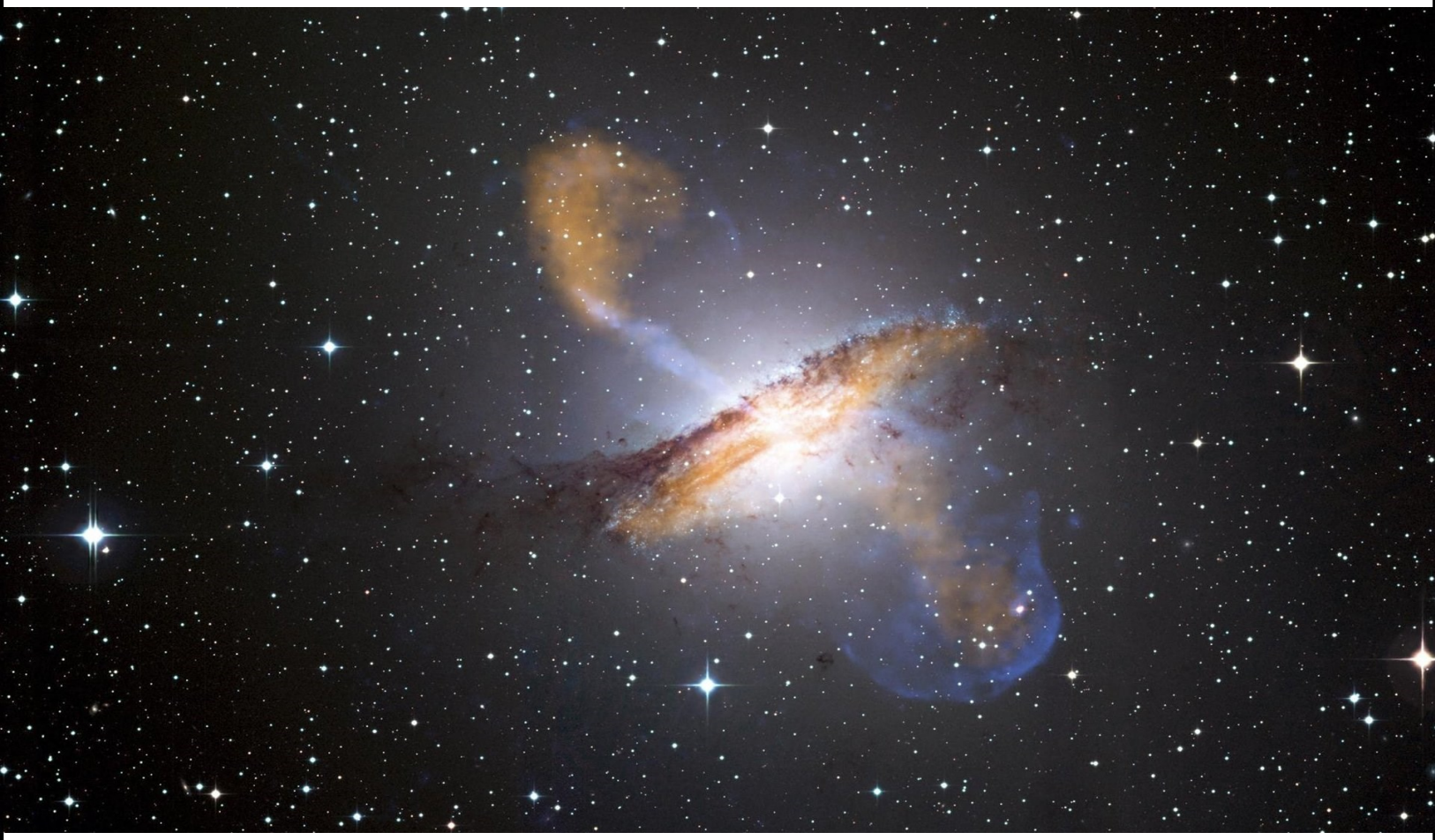

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