SHORT COMMUNICATION

A universal microscope manipulator

Peter S. Boyadzhiev[1], Tsanko S. Gechev[2] & Atanas D. Donev[1]

[1]Department of Zoology, University of Plovdiv "Paisii Hilendarski", 24 Tsar Asen Str., 4000 Plovdiv, Bulgaria. boyadz@uni-plovdiv.bg; atdonev@uni-plovdiv.bg
[2]Department of Plant Physiology and Molecular Biology, University of Plovdiv "Paisii Hilendarski", 24 Tsar Asen Str., 4000 Plovdiv, Bulgaria. tsangech@uni-plovdiv.bg

ABSTRACT. A universal microscope manipulator. A modified and improved model of a mechanical manipulator for observation of pinned and mounted insects is described. This device allows movement of the observed object around three perpendicular axes in the field of vision at all magnifications of stereomicroscopes. The main improvement of this new model is positioning of the guiding knobs for rotating around two of the axes next to each other, allowing faster and easier manipulation of the studied object. Thus, one of the main advantages of this device is the possibility to rotate the specimen without the need to refocus. The device enables easily reaching a precession deviation in the intersection point of axes up to 0.5 mm in the process of assembling.

KEYWORDS. Improved equipment; microscopy; mounted insects; specimen manipulation.

RESUMO. Um manipulador microscópico universal. Um modelo modificado e melhorado de um manipulador mecânico para observação de insetos fixados e montados é descrito. Este dispositivo permite o movimento do objeto observado em torno de três eixos perpendiculares no campo de visão para todas as ampliações do microscópio. A principal melhoria deste novo modelo é o posicionamento dos botões de rotação em torno dos dois eixos ao lado do outro, permitindo uma rápida e fácil manipulação do objeto estudado. Assim, uma das principais vantagens deste dispositivo é a possibilidade de girar o espécime sem a necessidade de mudar o foco. O dispositivo permite facilmente chegar a um desvio de precisão no ponto de interseção dos eixos de até 0,5 mm no processo de montagem.

PALAVRAS-CHAVE: Equipamento aperfeiçoado; insetos montados; manipulação de espécimes; microscopia.

Mechanical manipulators used in entomological studies facilitate significantly observations and exact measurements of insects and other small objects under stereomicroscopes. A considerable disadvantage of the earlier designs of Oliver (1969) and Köppen (1966) manipulators is the absence of rotation movement around the permanent vertical axis. This was corrected in the Lobanov & Kotjurgin (1975) and Boyadzhiev & Bozhinova (2006) designs but the handling in those models was more difficult as the knobs for rotating in different axes were rather distant. Furthermore, in the earlier manipulator a 'blind spot' occurs when the holding arm is positioned between the insect and the objective.

The main objective of this work was to design a new manipulator that is easy to handle, especially during the rotation and tilting of the specimen which can now be done with fewer manipulations and without refocusing in small magnification of stereomicroscope. When the position is changing the object also remain in the viewing area of the stereomicroscope in all magnifications. Achieving this, along with the development of solid flexible coupling inside the supporting arm, gives a significant advantage of the new manipulator over the previous design by Boyadzhiev & Bozhinova (2006) and allows more efficient manipulations while retaining the positive features of earlier designs.

The new manipulator corresponds better to the specific requirements for entomological studies of pinned and mounted insects. In addition, two modification of presented scheme are suitable for studying of bigger biological objects like small animals, crania of rodents, plant parts, or for studying under compound microscope, and is therefore a universal instrument that can assist any biologist who uses stereomicroscope or compound microscope.

The design of the manipulator provides object movement with three degrees of freedom with small precession displacement. The device presented here was developed following the methods for improved observation and measurements of pinned and mounted insects of Martin (1977) and Noyes (1982, 2011). In the last case, the mounted object is placed on card or point and simultaneously eccentrically toward the entomological pin axis. The proposed device (Fig. 1) consist of: a supporting arm [6] (two pieces of copper pipe, ø 6 mm, curved with tube bender and soldered with silver) with a rubber base [11] for holding an entomological pin [12]; a sheet steel base [3]; a sub-base [1] with screw [4], providing rotation of the base [3] 360° around its axis; a standard pointer [17] marking the axes-intersection reference point, mounted on supporting pillar [14]; a rotational spindle [10] mechanically assembled with a helical spring drive [9] and indirectly with a flexible steel core [8] (ø1.2 mm) inside the supporting arm that provides the accurate rotation of spindle when the knob [13] is turned.

The device has been designed so the spindle [10] and the sub-base screw [4] are par-axial in the position on Fig. 1: the insect is placed along this axis at a higher position above the rubber mount base [11]. The standard pointer [17] is used to determine the intersection point of axes. The final 30 mm of this pointer should be made of hair to avoid damaging of the examined object while the entomological pin is being positioning in the rubber base [11]. The supporting pillar [14] on which the standard pointer is mounted also supports a white screen [16] attached to an arm [15] capable of rotation and vertical movement below the specimen. This holder is restrained vertically, to prevent sample damage.

If the procedure of adjusting and fixing has been performed precisely, the insect can be rotated around the intersection point of axes while remaining in the field of vision in all magnifications of the stereomicroscope. This allows observation of the object in all possible positions with fewer manipulations. This is a significant advantage over the previous models. In the process of changing the position, defocusing is possible when the magnification is high by reason of the small depth of focus and volume of the object. The proposed manipulator has a reliable connecting mechanism for rotational movement and a simplified design allowing easy handling.

During the assembly process, one point must cross three axes. This is accomplished by using a stereomicroscope with an eyepiece micrometer and a test-object with size 0.5 mm. The test-object is mounted on a point and the latter is positioned at 12–13 mm from the top of the entomological pin – a standard length (Martin 1977). In assembly, first connect the holder [5] with the base [3] with screw [4] and add the knob [7] (Fig. 1). Next insert the supporting arm [6] into the neck of the knob [7] (functioning as a socket) so that small movements in position are possible, using porous matter between them. After this, insert the advance coupled drivers [8, 9, and 10] in the supporting arm [6], then connect the rubber base [11] to the spindle [10] and the core [8] to the knob [13], using cyanoacrylate adhesive. Following this, it is necessary to set the test-object above the axis of the spindle [10] so as to rotate around itself by turning the knob [13]. This is observed and calibrated using the eyepiece micrometer at low magnification when the knob is turned. After this, the test object must be also adjusted to intersect the axis of the knob [7]. This is easily achieved if the neck of the knob [7] has approximately 3 mm larger inner diameter in comparison with external diameter of the supporting arm [6]. That enables changing the position of the object relative to the axis of the knob [7] and thereby crossing the axis of the knob [7] to the axis of the spindle [10]. If necessary, small plastic deformation in distal part of the supporting arm [6] is also possible and that should allow another way for adjusting to cross the object with the axis of the knob [7]. Next, the base [3] and sub-base [1] are assembled and adjusted to be on axis with the test-object. For this purpose, the positions of the supporting arm [6] toward the knob [7] and the holder [5] toward the base [3] are changed in a suitable manner. The precess deviation while rotating the object around all of the axes should not be greater than 0.5 mm. The position between supporting arm [6] and knob [7] must be fixed using cyanoacrilate adhesive. We also used this method for fixing the position between holder [5] and base [3]. Finally, the supporting pillar [14] assembled with the standard pointer [17] and the white screen [16] is mounted to the steel base. The distal end of the pointer must within about 5 mm of the axes intersection point. Constructed in this way, the manipulator

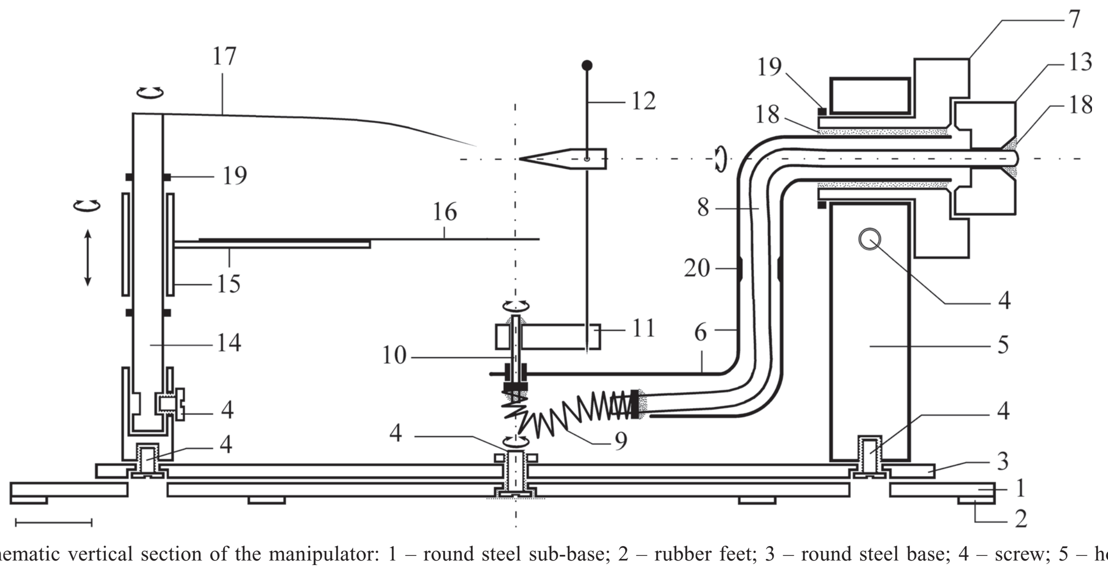

Fig. 1. A schematic vertical section of the manipulator: 1 – round steel sub-base; 2 – rubber feet; 3 – round steel base; 4 – screw; 5 – holder of the supporting arm; 6 – supporting arm; 7 – knob for rotating of the supporting arm; 8 – flexible steel core; 9 – helical spring; 10 – rotational spindle; 11 – rubber mount base; 12 – entomological pin; 13 – knob for rotating of the core; 14 – supporting pillar; 15 – screen arm; 16 – white (black) screen; 17 – standard pointer; 18 – porous matter impregnated with cyanoacrylate adhesive; 19 – stopper; 20 – silver solder. Scale line = 1 cm.

works successfully with almost all stereomicroscopes used in entomological research.

It is necessary to set the mounted insect at the intersection point of the axes for the correct use of the proposed device. This is easy to perform when all elements are set in the initial position (Fig. 2). Then the top of the standard pointer marks the intersection point of the axes 5 mm forward. This position can be found easily if during assembling it is marked on the already fixed pillar. The screen is turned farther to one side so as not to be an obstacle. One should hold up the rubber base [11] with two fingers of the one hand and stick the pin on the rubber base with the other hand, so that the insect stands 5 mm in front of the top of the pointer. The standard pointer can then be turned to one side so as not to be an obstacle. It is sufficient to rotate the knobs 7 and 13 completely to check the correctness of the fixing. If the fixing had been made correctly, the insect may be turned around completely at the intersection point of axes. If this is not possible, the pin must be re-fixed. In cases when the object is close to the intersection point, small changes in the position of the entomological pin over the rubber base can be made. After a little practice, the entomologist successfully learns how to set the insect at the intersection point of axes without using the pointer [17]. If an additional light source is available that allows using the white-coloured base [3] as screen, it's recommended dismantling the supporting pillar [14]. This will make work with manipulator absolutely safe for the observed insects.

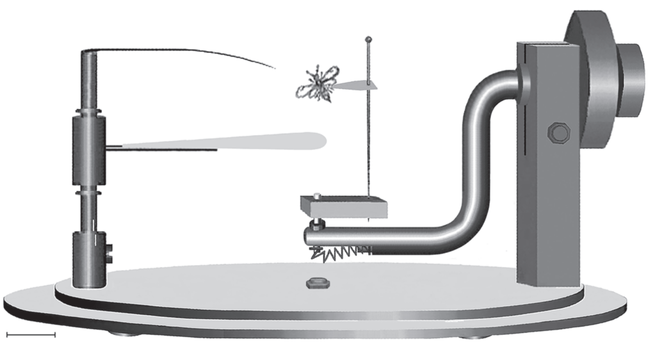

Fig. 2. Three-dimensional view of the manipulator. Scale line = 1 cm.

The proposed manipulator also fully satisfies the requirements for observation of pinned specimens. In this case, it's also recommended the advance dismantling of the supporting pillar [14] (Fig. 1). After that pin has to be fixed on the rubber base [11] near and parallel to the spindle [10]. This is enough to set the object at the intersection point.

All devices have been tested for alignment accuracy at the end of assemblage. For this purpose, a test-object (0.5 mm) mounted on point and fixed at a standard distance from the top of the entomological pin was used. The eyepiece measure scale was calibrated in advance with an object-micrometer. The precess deviation was measured at the highest magnification of the stereomicroscope when the test-object was at the intersection point. The test results showed that each manipulator has a precess deviation at the intersection point on the order of 0.5 mm. This deviation is perfectly sufficient even for observing and investigating some of the smallest insects with size 0.5–3 mm. The tests also showed that insects remain in the microscope's field of vision during rotation if the entomological pin is fixed correctly.

An important outcome of the test was the opportunity to manipulate only with one hand, because of the weight of the manipulator that provides stability and the good cohesion between the rubber legs and the stage. When the position is changed, small defocusing result from object volume and the small depth of focus are possible. This requires fine re-tuning of the stereomicroscope focus with the other hand of the entomologist.

One of the main requirements of the type specimen studies (especially holotypes and other type specimens) is to avoid any damage of the specimen during the observation. The proposed manipulator fully satisfies this requirement by the advance dismantling of the supporting pillar [14] (Fig. 1). Then the base performs the part of the screen for object observation. In this case, observation of the insect's transparent parts was accomplished in diffuse reflected light when the white-coloured base [3] was irradiated by an additional light source placed close to the stereomicroscope. Using the proposed manipulator, the time for observation and determination was shortened considerably by the quick and exact adjustment of the insect in the field of vision at the right angle of view. In addition, the specimens were safeguarded from accidental damage in the entire process of investigation.

The schematic diagram presented is the result of three year experiments (2007–2010) conducted by the first author, creating the optimum mechanical manipulator for faunistic and taxonomic study of small insects, mounted on cards or points. Three working devices have been constructed by the authors. More than 3000 specimens from different Hymenoptera species were used by the authors for taxonomic and faunistic investigations, including about 150 type specimens from the family Eulophidae (Hymenoptera, Chalcidoidea). The calibrating results came as a result of these measurements. In addition, the prototypes were tested by many participants of Seventh International Congress of Hymenopterists, in Köszeg, Hungary (20–26 June 2010).

Using the ideas presented in this paper, many variations are possible. For objects larger than 3 cm, manipulators of larger dimensions may be constructed by increasing the length of the supporting arm [6] and a proportional increase in the dimensions of the base, sub-base and the holder of the supporting arm. Such modification for different zoological objects was constructed and tested especially for measuring of crania of small rodents. This modification has two degrees of freedom, longer supporting arm and small aluminium clip which is solidly assembled toward rubber base of the supporting arm (Fig. 3A). Another smaller device was constructed and tested for working under Carl Zeiss Amplival microscope (Fig. 3B). Its rectangular-shaped base is suitable for stable fixing toward mechanical stage of the compound microscope. Using reflected light from lateral source it is possible to obtain images with higher quality and magnification up to 640 times (highest magnification of most stereomicroscopes is about 100 times). Thus preparation of microscope slides is avoided in some cases. Two comparative photography of stigmal vein of forewing of *Puklina asphodelinae* Boyadzhiev, 2003 (Hymenoptera, Eulophidae) taken from specimens prepared on microscope slide (Fig. 4A) and on point (Fig. 4B) are presented. The last method was previously used by the senior author for description of male antenna and forewing of *Neotrichoporoides bulgaricus* Graham, 1987 (Yefremova *et al.* 2010).

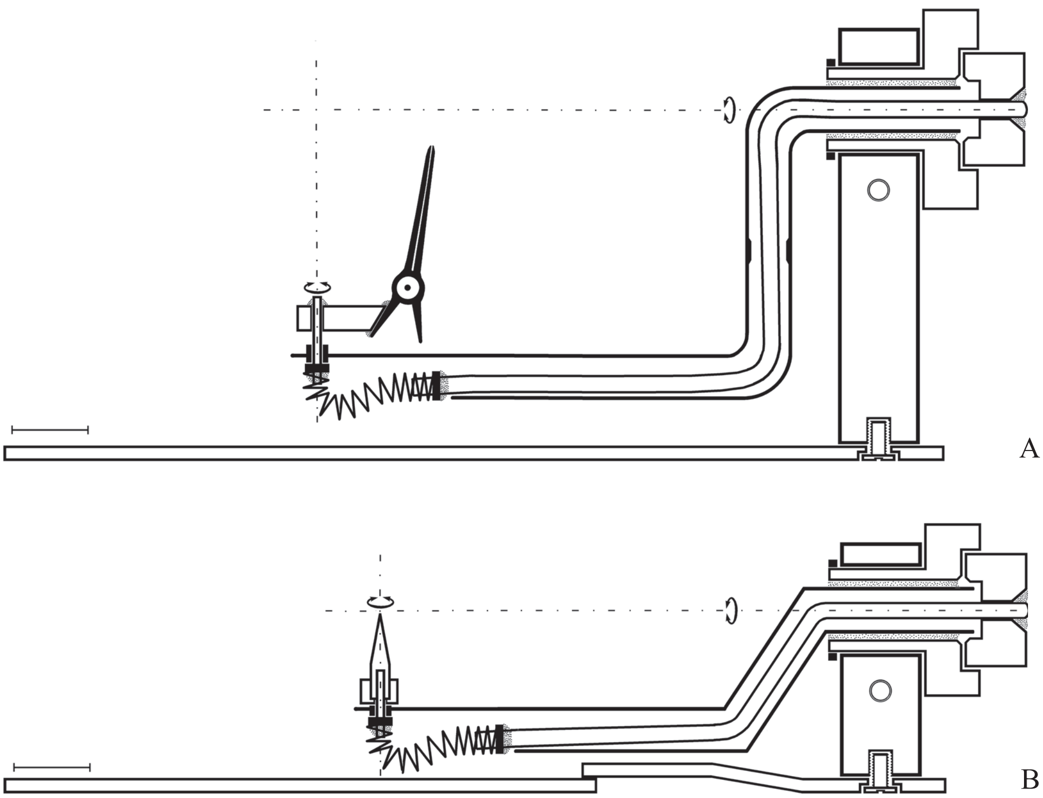

Fig. 3. A schematic vertical section of modified versions of the manipulator: A – for studying of larger biological objects. The self-closed aluminium clip is solidly assembled toward rubber base; B – for studying under Carl Zeiss Amplival microscope. The rubber base has a small bed for point. Scale lines = 1 cm.

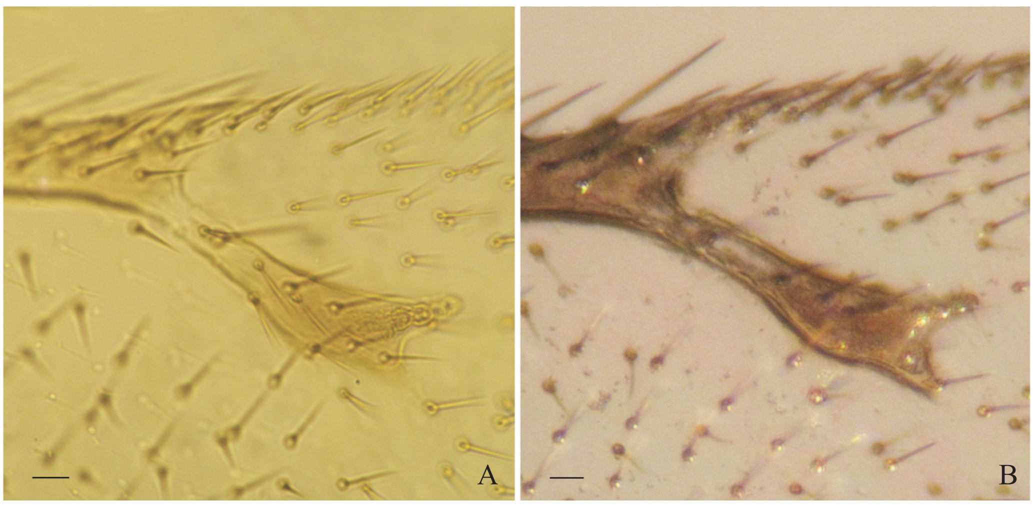

Fig. 4. Comparative photography of stigmal vein of female forewing of *Puklina asphodelinae* taken by Canon Power Shot SD990 IS digital camera under a Carl Zeiss Amplival microscope: A – from specimen mounted on microscope slide using transmitted light; B – from specimen mounted on point (Paratype) using reflected light from lateral source. Scale lines = 10 µm.

The constructed prototypes of the manipulator are available at the Department of Zoology, University of Plovdiv, Bulgaria and can be used by any visiting colleague upon request. The authors will be grateful for any feedback from colleagues, which can further improve the manipulator.

The proposed new manipulator is easy to use and allows observation of the object in all possible positions with fewer manipulations. It also allows the entomologist a simple and exact adjustment of insects in the right position for observation and at the same time ensures the maximum security of the objects being investigated. The device facilitates entomologists in their investigation of small insects and reduces the time for adjusting and measuring. In addition, two versions of the manipulator were developed for studying under compound microscope or for different biological objects like crania of rodents or plant parts, thus making the manipulator a universal instrument that can facilitate microscope observations.

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